INVESTIGATION OF ACETYLSALICYLIC ACID BIOAVAILABILITY AND ITS EFFECT ON PROSTAGLANDIN-$F_{2\alpha}$ IN DOGS

by

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INTRODUCTION

Salicylic acid was first obtained from leaves and barks of the willow tree as the glycoside, salicin. Its acetic acid ester, acetyl salicylic acid (aspirin), was first synthesized by Von Gerhardt in 1853. The name, aspirin, was derived from the terms "acetyl" and "spirsaure", the latter referring to the genus Spiraea, one of the botanical sources of salicylic acid[1].

Aspirin is prepared by direct acetylation of salicylic acid with acetic anhydride using a small amount of sulfuric acid as catalyst[2]. Aspirin is generally available as a powder and was introduced into medicinal practice in Germany before the turn of the century. The tablet form was introduced into the United States in 1915. It has since become the most widely used analgesic, antipyretic, and anti-inflammatory drug in the world[3]. Recent experimental and clinical studies[1] suggest aspirin may have antithrombotic properties, preventing cerebro vascular thromboembolism and myocardial infarction, and therefore reducing the incidence of stroke and heart attack. The therapeutic effects of aspirin are apparently due to its inhibitory effect on the synthesis of prostaglandins which act as mediators of pains and fevers[4].

Because aspirin is so readily available, it is also easily abused. Aspirin is the most common cause of drug poisoning in young children[5]. Aspirin is more toxic to the gastric mucosa of man and animals than its parent compound, salicylic acid[6,7]. Aspirin ingestion causes peptic ulcer[8]; doses of 1-3 g/day induces occult gastro intestinal bleeding in about 70% of normal subjects[1]. Aspirin intoxication results in respiratory alkalosis and metabolic

acidosis[9], hyperglycemia or hypoglycemia[5,9], and not infrequent death.

The objective of this study was to investigate: (a) the in vitro and in vivo bioavailability of several popular commercial sources of aspirin; and (b) the effect of aspirin on endofeneous prostaglandin-$F_{2\alpha}$ of dogs.

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THE IN VITRO AND IN VIVO AVAILABILITY OF COMMERCIAL ASPIRIN IN DOGS

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SUMMARY

Bioavailability of eleven commercial brands of aspirin was assessed in vitro using simulated gastric juice and also in vivo in dogs. Gastric juice and salicylate concentrations were measured by fluorescence spectrophotometer. Excedrin was dissolved fastest of all the brands of aspirin, followed by Ascriptin; the slowest was Excedrin PM. In dogs dosed orally with Excedrin or Ascriptin, total plasma salicylate peaked about 2.5 hrs after dosing with Excedrin and 3 hrs post dosing with Ascriptin. About 96% of the salicylate was bound to plasma proteins. The rate at which Excedrin leaves the plasma is different from that of Ascriptin. Plasma salicylate concentration from Excedrin droped to an average of 12 mg/ml in 6 hrs while Ascriptin peaked and remained virtually at that level for more than 12 hrs.

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The salicylates, which include salicylic acid, its esters, and other derivatives, have been used for many years to treat fevers and rheumatism. They were first obtained from willow trees which contain salicin -- a glycoside of salicylic acid. One of the most widely used derivatives of salicylic acid is acetyl salicylic acid (aspirin). Aspirin is prepared directly by the acetylation of salicylic acid with acetic anhydride using a small amount of sulfuric acid as catalyst[1].

Aspirin is used as an analgesic, anti-rheumatic, antipyretic and anti-inflammatory. Recent experimental and clinical studies[2] suggest aspirin may have antithrombotic properties, preventing cerebrovascular thromboembolism and myocardial infarction, and therefore reducing the incidence of stroke and heart attack.

Aspirin is given mainly by oral administration. It is rapidly absorbed for the most part from the stomach and upper small intestine; only a small amount is absorbed in the lower intestinal tract[1]. Aspirin is a moderately strong acid (with a pKa of 3.5), so its gastric absorption is pH dependent[3]. Aspirin is absorbed in the unionised form and is lipid soluble. Its rate of fecal excretion is therefore lowered in the acidic environment of the stomach[4,5]. The rate of absorption of aspirin depends on the rate of dissolution. The dissolution rate of aspirin was found to be independent of the crystal growth rate, salicylic acid content (up to 3.9% w/w), habit anf particle size[6].

Aspirin circulates in the blood mostly bound to plasma protein with a little bound to erythrocytes. Aspirin is bound more

strongly to human serum albumin (HSA) than is salicylic acid[7]. The peak level of aspirin is reached within two hours, but varies with the dose of aspirin given, and species of animal[8].

The purpose of this study was to examine the dissolution rate of 11 commercial sources of aspirin in artificial gastric juice and then to determine the bioavailability in dogs of the two most soluble brands. No previous study of commercial aspirin bioavailability in dogs has been published.

MATERIALS AND METHODS

We placed intact tablets of eleven different commercial aspirin preparations in artificial gastric juice. The commercial brands of aspirin used were Arthritis Pain Formula 7.5 grs; Arthritis Strength Bufferin 7.5 grs; Ascriptin 5 grs; Bayer Aspirin 5 grs; Bufferin 5 grs; Emperin 3.5 grs; Excedrin 3 grs; Excedrin P.M. 3 grs; Parke Davis Aspirin 5 grs; Purity Aspirin 5 grs; and Squibb Aspirin 5 grs. The equivalent of 5 grs (325 mg) of each brand was used for the in vitro study. The gastric juice were incubated in a shaker water bath at 37 C. Six samples of the gastric juice were drawn at 0 time and at 30 minutes intervals until the concentrations stabilized, and the salicylate content was measured in a fluorescence spectrophotometer[a,5,6]. The fluorescence emission and excitation spectra were measured at wavelengths of 468 and 390 nm, respectively.

The two most soluble brands of aspirin (Ascriptin and Excedrin) were used for the in vivo tests with two female shepherd dogs. One weighed 7.3 kg, and the other 20.5 kg. The dogs were off feed for 24 hrs before dosing and throughout the duration of each experiment. The dogs were given a single oral dose of 30 mg of salicylate/kg body weight. This dose was based on the recommendation of 25-35 mg salicylate/kg[8], and was repeated two weeks later with the other brand. Heparinized blood was collected at 0 time and at 30 minutes intervals for 12 hrs after each administration. Blood samples were centrifuged immediately at 27,000g for 20 minutes and the plasma was separated and

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[a]Perkin-Elmer Fluorescence Spectrophotometer model MPF 44A

frozen for analysis[9,10].

Salicylate binding to plasma proteins was determined in all the plasma samples collected[11,12]. Stirred ultrafiltration cells with a volume capacity of 3 ml[b] and pellican membranes with a nominal molecular weight limit of 25,000[b] were used to separate free and bound salicylate. Two and a half milliliters (2.5 ml) of plasma was filtered at 37 C by the pressure of 40 psi of medical grade nitrogen; 0.1 ml of the filtrate was collected. The salicylate concentration of the filtrate and the parent solution before and after filtration was determined. The percentage of bound salicylate was calculated after correcting for non-specific losses by dividing the salicylate concentrates after filtering by the total salicylate concentration in the plasma before filtration. The measured fluorescences were converted to units of ug/ml by a standard curve. The standard curve was linear up to a concentration of 2.0 ug/ml.

Preparation of gastric juice

The artificial gastric juice was prepared by dissolving 2 g sodium chloride (NaCl) and 3.2 g of pepsin powder[c] separately in glass distilled water and then mixing the resulting solution together in a 1 l volumetric flask. Concentrated hydrochloric acid (7.0 ml) was added before bringing the final mixture to 1 l final volume with glass distilled water[13].

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[b]Cat #PSED 01310, Millipore Corp, Bedford, Mass.

[c]Sigma Chemical Company, St. Louis, Mo.

RESULTS

The dissolution and hydrolysis of the various brands of aspirin to salicylic and acetic acids in artificial gastric juice is shown in Figures 1 and 2. The concentration of salicylate increased gradually with time in all samples until a constant level was reached. Excedrin produced the highest concentration of salicylic acid in the solution at the fastest rate. It reached a peak concentration of 16.4 mg/250ml of solution in 2 hrs. Excedrin was followed by Ascriptin and Arthritis Strength Bufferin, which each produced 12.3 mg salicylate/250 ml. Excedrin P.M. gave the least concentration (5.8 mg/250 ml) with a peak concentration about 7 hrs. The remaining seven brands reached their peaks in 6-10 hrs and their concentrations in solution varied from 7.8 to 9.8 mg salicylate/250 ml solution.

Excedrin and Ascriptin were used for the in vivo study in dogs. The Excedrin gave the same pattern of bioavailability as observed in the in vitro study. It peaked in the blood in an average of 2.5 hrs, only 30 minutes later than the artificial gastric juice (Figure 3). Ascriptin peaked between 2 and 4 hrs in the two dogs, and much earlier than in the in vitro study (Figure 4).

The percentages of salicylate found protein bound to each dog's plasma at each sampling time are summarised in Tables 1 and 2. Ascriptin was bound more to dog plasma protein (92.3% in dog A and 97.5% in dog B) than Excedrin during peak concentration of salicylate in the blood. Excedrin-dosed dogs A and B bound just 89.3 and 56.3% to their plasma protein, respectively, during peak blood salicylate concentration.

DISCUSSION

The rate of availability of salicylic acidin vitro and in vivo depends on the rates of disintegration, dissolution and hydrolysis of acetyl salicylic acid (aspirin) to salicylic and acetic acids. Acetyl salicylic acid is hydrolysed by aspirin esterase in blood plasma within 20 to 30 minutes, but the disintegration rate of an uncoated tablet depends on the compression pressure, binders, lubricants, and the amount of disintegrators added[14]. Rates of dissolution of commercial aspirin depend on thermodynamic activity rather than on crystal growth rate, salicylic acid content (up to 3.9% w/w), habit or particle size[6]. Differences in the concentration of salicylic acid released from each of the 11 brands of aspirin could be due to thermodynamic differences (affecting the rates of dissolution and disintegration) among the formulations[15]. Other causes of variation in salicylic acid concentration among the formulations could relate to the relative content of an immunogenic impurity, acetyl salicylic anhydride, in the tablets[16]. Acetyl salicylic anhydride which forms N-salicylol in solution, might reduce the amount of salicylate in solution.

In the in vivo study, Excedrin, which peaked between 2-3 hrs, was biologically available faster than Ascriptin (Figures 3 and 4). The rate at which the salicylate from Excedrin leaves the plasma of dog A was faster than in dog B (see Figure 3). Ascriptin which peaked between 2-4 hrs, maintained relatively high salicylic acid concentration in the plasma of both dogs for 24 hrs.

Our results agree with earlier findings that peak plasma concentration of salicylic acid occur between 2-4 hrs; after which the concentration gradually drops until it is almost zero at 24 hrs[8,17,18].

The data on the percentages of salicylic acid bound to protein suggest that as the plasma salicylate concentration increased, the percent bound to protein also increased until all sites for salicylic acid on the protein are occupied or have reached equilibrium. Then the percent bound remained virtually the same until the plasma concentration of the free salicylic acid started decreasing due to metabolism and excretion. The salicylate bound to plasma protein is gradually released to replace that portion being metabolised. At low concentration of plasma salicylate (dog A, Table 1 and 2), the percent salicylic acid bound to plasma protein was low and may have been subject to sampling error. On the average, about 96% of the salicylates was bound to the plasma protein of the two dogs. This agrees with earlier findings that salicylates are tightly bound to plasma protein, particularly to albumin[7,19].

Slight variations in the time of peak salicylate blood levels in the two dogs may have been due to variation in particle size of the tablets used and to individual animal variation. This may have resulted from different pH environment of gastric absorbing sites[3] and different body mass[1]. The varying plasma salicylate concentrations 4 hrs after dosing also showed that the rates of metabolism and kidney excretion of the two brands varied between the two dogs.

Figure 1.

Concentrations of salicylate from six different commercial preparations of aspirin in simulated gastric juice.

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

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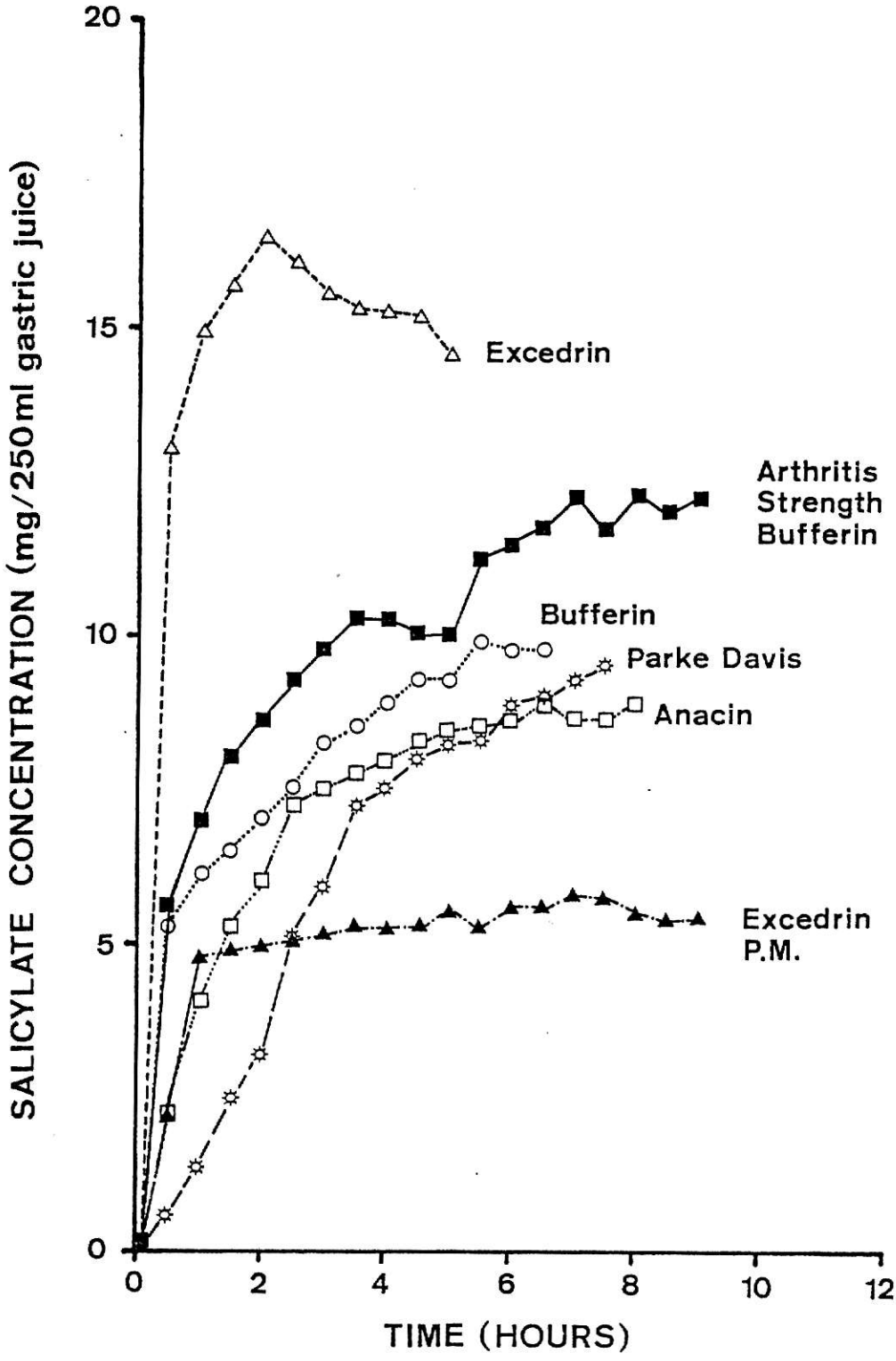
SALICYLATE CONCENTRATION (mg/250ml gastric juice)
20
15
10
5
0
0
2
4
6
8
10
12
TIME (HOURS)
Excedrin
Arthritis Strength Bufferin
Bufferin
Parke Davis
Anacin
Excedrin P.M.

Figure 2.

Concentrations of salicylate from five different commercial preparations of aspirin in simulated gastric juice.

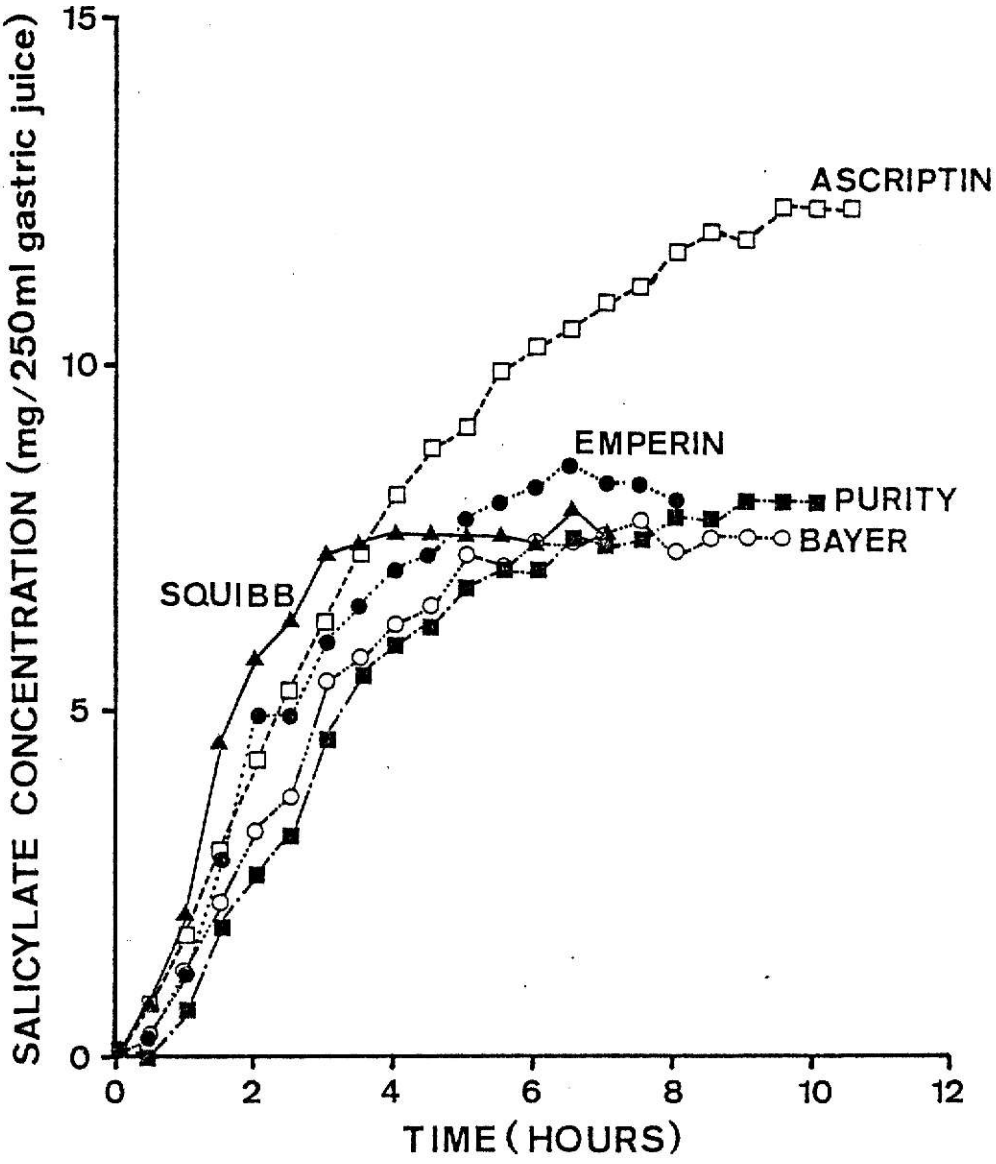
SALICYLATE CONCENTRATION (mg/250ml gastric juice)
15
10
5
0
0
2
4
6
8
10
12
TIME (HOURS)
ASCRIPTIN
EMPERIN
PURITY
BAYER
SQUIBB

Figure 3.

Salicylate concentrations in blood plasma of dog A (- - -) and dog B (———) after single doses of 30 mg/kg of Excedrin.

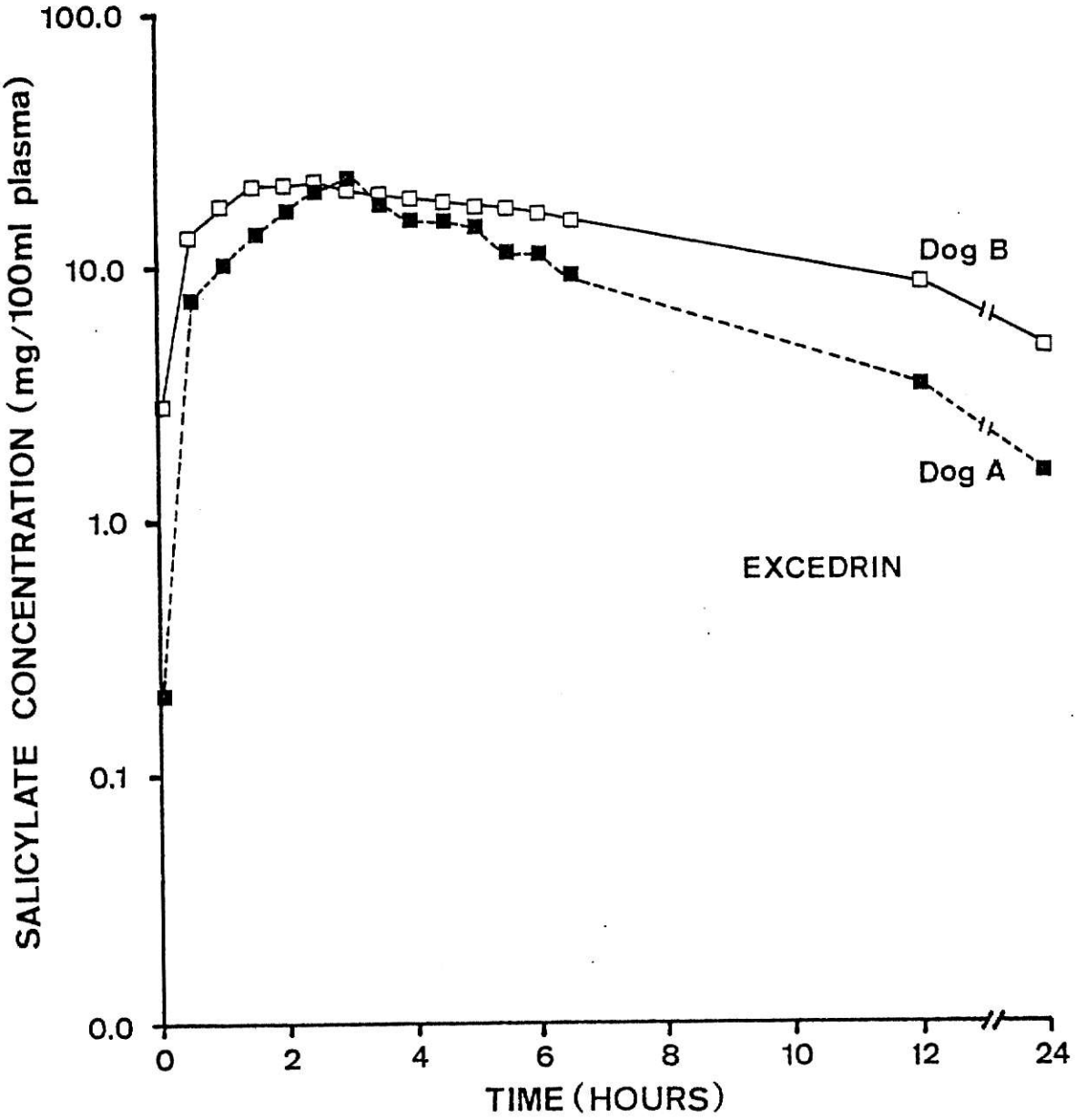
100.0
10.0
1.0
0.1
0.0
SALICYLATE CONCENTRATION (mg/100ml plasma)
0
2
4
6
8
10
12
24
TIME (HOURS)
Dog B
Dog A
EXCEDRIN

Figure 4.

Salicylate concentrations in blood plasma of dog A (- - -) and dog B (———) after single doses of 30 mg/kg of Ascriptin.

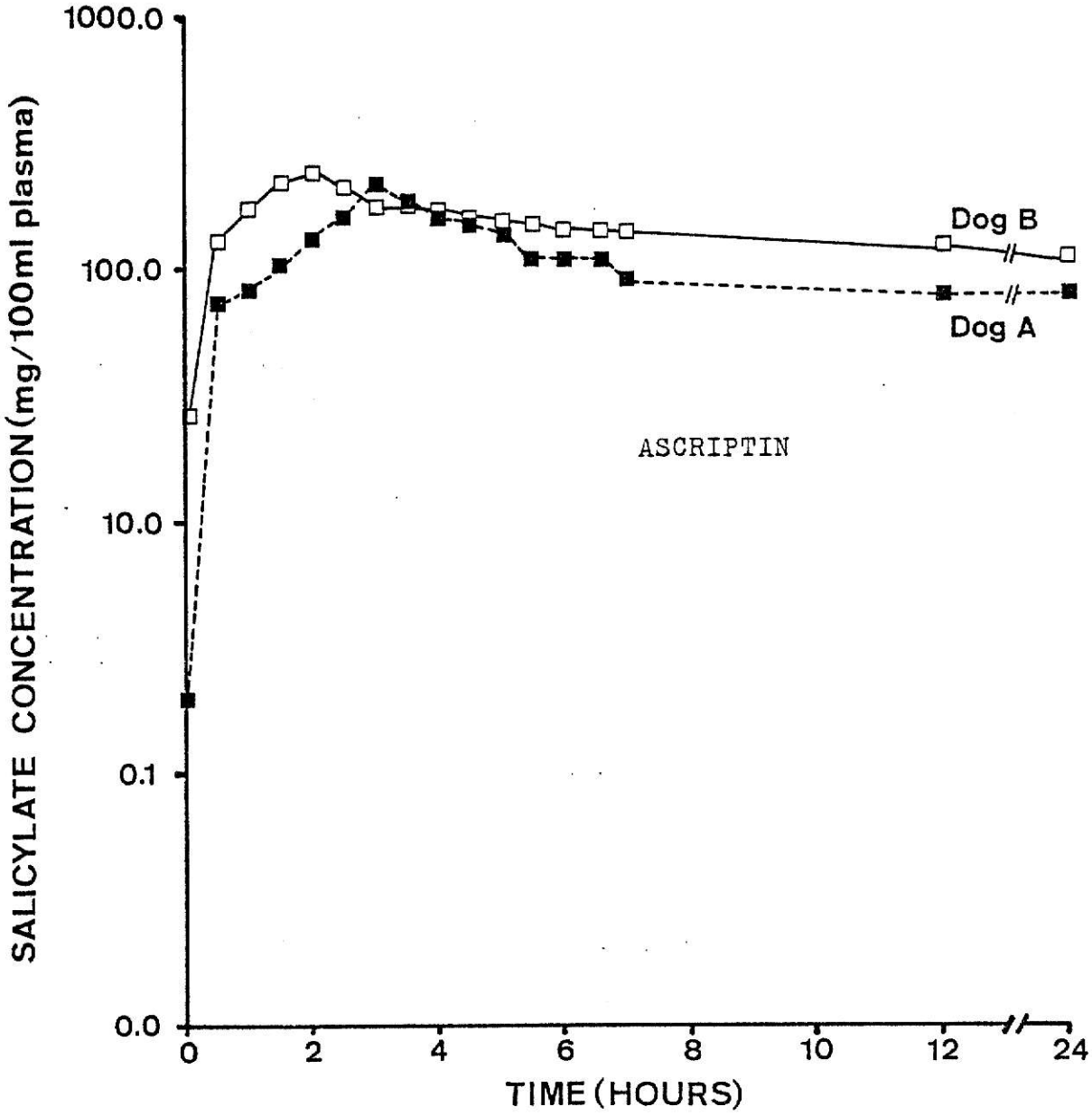
1000.0
100.0
10.0
0.1
0.0
SALICYLATE CONCENTRATION (mg/100ml plasma)
Dog B
Dog A
ASCRIPTIN
0
2
4
6
8
10
12
24
TIME (HOURS)

Table 1. Total salicylate concentration and percent protein bound in blood plasma of two dogs after a single 30 mg salicylic acid /kg of body weight dose of Ascriptin

Time (hrs)	Dog A		Dog B	
	Total conc. (mg/100ml)	Protein bound (%)	Total conc. (mg/100ml)	Protein bound (%)
0.0	7.5	93.3	2.1	91.6
0.5	9.4	93.6	6.9	91.6
1.0	9.5	93.0	12.3	78.3
1.5	12.5	92.0	12.8	74.8
2.0	10.7	93.5	19.2	97.5
2.5	11.3	88.5	17.1	62.6
3.0	9.4	85.1	15.5	77.9
3.5	16.8	92.3	12.9	76.8
4.0	13.6	95.6	11.0	67.3
4.5	10.7	95.3	9.2	70.8
5.0	11.7	94.0	7.7	76.0
5.5	11.3	92.9	11.6	68.2
6.0	10.0	90.0	11.3	77.6
6.5	11.5	91.3	15.8	74.6
7.0	9.5	94.7	8.3	75.7
7.5	10.8	92.6	19.2	84.1
8.0	9.6	93.8	8.0	79.6
8.5	8.1	92.6	8.0	71.7
9.0	10.7	93.5	5.0	77.6
9.5	9.1	93.4	6.9	81.3
10.0	10.9	96.3	3.5	75.0

Table 1 cont'd. Total salicylate concentration and percent protein bound in plasma of two dogs after a single 30 mg salicylic acid/kg body weight dose of Ascriptin

	Dog A		Dog B	
Time (hrs)	Total conc. (mg/100ml)	Protein bound (%)	Total conc. (mg/100ml)	Protein bound (%)
10.5	11.6	94.8	10.5	74.1
11.0	7.9	94.9	9.8	76.4
11.5	9.8	96.9	4.0	75.6
12.0	8.5	94.1	8.6	83.5
24.0	7.0	94.3	6.0	78.3

Table 2. Total salicylate concentration and percent protein bound in blood plasma of two dogs after a single 30 mg salicylic acid/kg of body weight dose of Excedrin

Time	Dog A		Dog B	
	Total conc.	Protein bound	Total conc.	Protein bound
(hrs)	(mg/100ml)	(%)	(mg/100ml)	(%)
0.0	0.2	50.0	2.8	92.9
0.5	7.2	83.3	13.2	98.5
1.0	10.4	83.9	17.5	88.6
1.5	13.6	91.9	20.6	86.5
2.0	16.6	86.0	20.8	86.5
2.5	20.0	90.1	21.4	88.8
3.0	22.4	89.3	18.8	85.3
3.5	18.0	95.8	18.5	89.2
4.0	15.8	88.5	18.1	85.6
4.5	15.4	93.8	17.4	89.3
5.0	14.4	94.9	16.9	86.4
5.5	11.2	92.3	16.4	91.7
6.0	11.2	91.2	15.1	89.4
6.5	9.0	93.8	15.1	89.4
12.0	3.4	64.7	8.0	95.8
24.0	1.5	60.0	4.7	95.3

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THE EFFECT OF ASPIRIN ON ENDOGENEOUS PROSTAGLANDIN-$F_{2\alpha}$ IN DOG PLASMA

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SUMMARY

Aspirin relieves pains and fevers by influencing the level of endogeneous prostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$). We determined the effect of two brands of aspirin on the concentration of plasma $PGF_{2\alpha}$ in two dogs over a 24-hr period using radio-immunoassay. Both brands of aspirin, in single doses of 30 mg/kg, reduced $PGF_{2\alpha}$ in the plasma. Lowest concentration of $PGF_{2\alpha}$ occurred 2 and 3 hrs after oral administration of Excedrin and Ascriptin, respectively. The percent free $PGF_{2\alpha}$ increased linearly up to a maximum of 85% free $PGF_{2\alpha}$. The percent free $PGF_{2\alpha}$ decreased as total $PGF_{2\alpha}$ concentrations decreased.

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The therapeutic properties of aspirin are due to its inhibitory effects on prostaglandin synthetase. The precursor of prostaglandin, arachidonic acid, is produced when tissue lipases or cell membrane phospholipases are activated. Aspirin inhibits the activity of prostaglandin synthetase in vitro and reduces biosynthesis of prostaglandin in a variety of systems[1]. Prostaglandins act as mediators of pain, fever, anaphylaxis, platelet aggregation and inflammation in the body. The activation of tissue lipases and cell membrane phospholipases results from cellular injury or cell toxins released from lysosomes[2]. A correlation has been reported between the potencies of anti-inflammatory drugs as inhibitors of prostaglandin synthetase in vitro and their peak concentrations in plasma after therapeutic dosage[3]. Prostaglandins are produced by many organs and are released spontaneously from numerous sites[4]. Aspirin significantly reduced potentiation of paw edema after concurrent administration of carrageenan and arachidonic acid[5].

There are different types of prostaglandins, including PGE, PGA and $PGF_{2\alpha}$. PGE and $PGF_{2\alpha}$ receptors represent two different macromolecular entities with a relatively small size difference between them[6]. Administration of $PGF_{2\alpha}$ raised blood levels of luteinizing hormone and testosterone[7], and increased secretion of estradiol[8], but it also inhibited milk ejection by a central block on oxytocin release[9]. There is individual age variation among sheep in the metabolism of $PGF_{2\alpha}$: maternal cotyledons are more active than fetal cotyledons[10]. Inhibition of prostaglandin synthesis by aspirin is due to acetylation of an active site of

prostaglandin synthetase. That site is the seryl residue at the $-NH_2$ terminus of the enzyme[11].

The purpose of this study was to determine the effect of two popular brands of aspirin on the $PGF_{2\alpha}$ content of dog plasma.

MATERIALS AND METHOD

The primary standard, dinoprost tromethamine ($PGF_{2\alpha}$, purity 99.8%), rabbit antibody and anti-rabbit gamma globulin (goat antisera) at dilutions of 1:10 and 1:3, respectively, and normal rabbit serum (NRS, dilutions of 1:100) for rabbit antibody diluent, were provided by Upjohn Company[a]. Trizma HCl and Trizma base were obtained from Sigma Company[b]. Tritium-labelled prostaglandin (^3H-$PGF_{2\alpha}$) was purchased from Amersham Corporation[c].

From the stock solution (1:10) of rabbit antibody, a dilution of 1:400 was prepared using NRS. The anti-rabbit gamma globulin (1:3) was diluted 1:20 with tris buffer.

Preparation of 0.05M tris buffer

Tris buffer was prepared by dissolving 4.44 g of Trizma HCl and 2.65 g of Trizma base separately in deionized water. The two solutions were mixed and brought to a final volume of 1 l. The tris buffer was adjusted to pH 8.0 with small amounts of Trizma base.

Sample collection

Blood samples were collected from two healthy female dogs weighing 12.7 and 21.4 kg, respectively, at 0, 0.25, 1.0, 1.5, 2.0, 2.5, 3.0, 6.0, 12.0, 18.0, and 24.0 hrs after oral administration of single dose of Excedrin and Ascriptin brands of aspirin at a dosage of 30 mg salicylic acid/kg body weight.

[a]Upjohn Company, Kalamazoo, Michigan.

[b]Sigma Chemical Company, St. Louis, Mo.

[c]Amersham Corporation, Arlington Heights, Il.

The two brands of aspirin were given one week apart. All food was removed from the dogs 24 hrs before dosing. Blood samples were immediately centrifuged at 2700 g for 20 minutes, the plasma separated, and used promptly for the radioimmunoassay analysis.

Radioimmunoassay of plasma

Sets of tubes were labelled control, unknown, standard or total count. We added 0.5 ml of NRS to the total count tubes, 0.5 ml tris buffer to control tubes, or 0.5 ml rabbit antibody to the unknown and standard tubes. To the control and total count tubes, 0.1 ml of tris buffer was added, while the standard and unknown tubes had 0.1 ml of dinoprost or 0.1 ml blood plasma added, respectively. Then 0.1 ml of ^{3}H-$PGF_{2\alpha}$ was added to all the tubes. Each tube was mixed thoroughly on a vortex mixer and allowed to equilibrate for 1 hr at room temperature.

After equilibration, 0.1 ml of tris buffer was added to the total count tubes, while 0.1 ml of anti-rabbit gamma globulin was added to the remaining three sets of tubes. The contents of these tubes were mixed and equilibrated for 24 hrs at 4 C. All the tubes were then centrifuged at 3500 rpm for 30 minutes at 4 C using a Sorvall RC 5B refrigerated superspeed centrifuge. A 0.5 ml aliquot was withd awn from each tube, placed in a scintillation counting vial with 15 ml of counting cocktail (Biosolv, Beckman formula BBS-3) and counted for radioactivity for 10 minutes. Standard and unknown samples were assayed in duplicate and mean values determined in disintegration per minute (dpm). The concentration of $PGF_{2\alpha}$ present at each time interval was obtained from a standard curve.

Calculations

The dpm for each tube was calculated as:

$$dpm = (CPM(tube) - CPM(control))/E$$

where CPM and E are counts per minute and instrument's efficiency (%), respectively. Percent free $PGF_{2\alpha}$ was determined as:

$$\%\text{Free } PGF_{2\alpha} = dpm(100)/dpm(\text{total count tubes}).$$

Mean dpm of total count tubes was 1,301,202.

RESULTS

The effect of Excedrin and Ascriptin on plasma $PGF_{2\alpha}$ is presented in Figures 1 and 2. Both brands decreased endogeneous $PGF_{2\alpha}$. The initial levels of endogeneous $PGF_{2\alpha}$ in the two dogs were different for the Excedrin and Ascriptin trials; respectively, 1400 and 700 pg/0.1 ml in dog A and 760 and 700 pg/0.1 ml in dog B. The initial $PGF_{2\alpha}$ levels decreased to minimum levels at about 2 hrs for Excedrin and about 3 hrs for Ascriptin. The $PGF_{2\alpha}$ levels did not fully return to pretreatment levels in 24 hrs. Ascriptin gave the lowest concentration of $PGF_{2\alpha}$, 5 and 1.9 pg/0.1 ml in dogs A and B, respectively. Ascriptin also lowered $PGF_{2\alpha}$ for a longer time than Excedrin.

Figures 3 and 4 show the percents of free $PGF_{2\alpha}$ as related to the total plasma concentrations of $PGF_{2\alpha}$. Both relationships are linear to about 85% free $PGF_{2\alpha}$.

DISCUSSION

The initial decrease in the endogeneous $PGF_{2\alpha}$ concentrations followed by a gradual increase to almost the initial concentrations reflects aspirin's inhibition of either the synthesis or release of $PGF_{2\alpha}$. Neff[13] similarly found that plasma radioactivity of $PGF_{2\alpha}$ was highest at the first 15 minutes post treatment, and nearly undetectable after 8 hrs. The inhibitory effect of aspirin on $PGF_{2\alpha}$ syntheis in various biological systems has also been reported[12]. Aspirin appeared to stop the synthesis of new $PGF_{2\alpha}$, causing the initial concentrations of $PGF_{2\alpha}$ to decrease until plasma concentrations of aspirin was reduced to levels that no longer affected $PGF_{2\alpha}$. New $PGF_{2\alpha}$ is synthesized at a very fast rate[13]. The lowest concentrations of $PGF_{2\alpha}$ were at 2 or 3 hrs after the administration of Excedrin or Ascriptin, respectively. These times corresponded with the periods of peak aspirin release following single oral therepeutic doses of these same aspirin brands in dogs[d].

The significant decrease in $PGF_{2\alpha}$ levels show that our single therapeutic dose of aspirin (30 mg/kg) is sufficient to cause inhibition of prostaglandin synthetase[12]. The levels of $PGF_{2\alpha}$ in the dogs were presumably different because of individual variation[14].

The percent free $PGF_{2\alpha}$ decreased from 89.8 to 56.1 pg/0.1 ml in dog A and 85 to 40 pg/0.1 ml in dog B due to the depleting effect of aspirin on the $PGF_{2\alpha}$, before increasing again 2-3 hrs

[d]Owonubi MO, Oehme FW: The in vitro and in vivo availability of commercial aspirin in dogs. Submitted to JAVMA, 1980

(Tables 1 and 2). The relationship between percent free $PGF_{2\alpha}$ and plasma $PGF_{2\alpha}$ concentration (Figures 3 and 4) is linear to 85% free $PGF_{2\alpha}$. This agrees with other workers[13,14] who found the linear part of the curve between 20-80% free label. This spanned 10-15 fold changes in concentrations. Non-linearity of the curve at free $PGF_{2\alpha}$ greater than 85% may be due to the saturation of $PGF_{2\alpha}$ binding sites on the rabbit serum antibodies.

Figure 1. Prostaglandin-$F_{2\alpha}$ concentrations in blood plasma of dog A (- - -) and dog B (———) after a single dose of 30 mg salicylic acid/kg of body weight as Excedrin

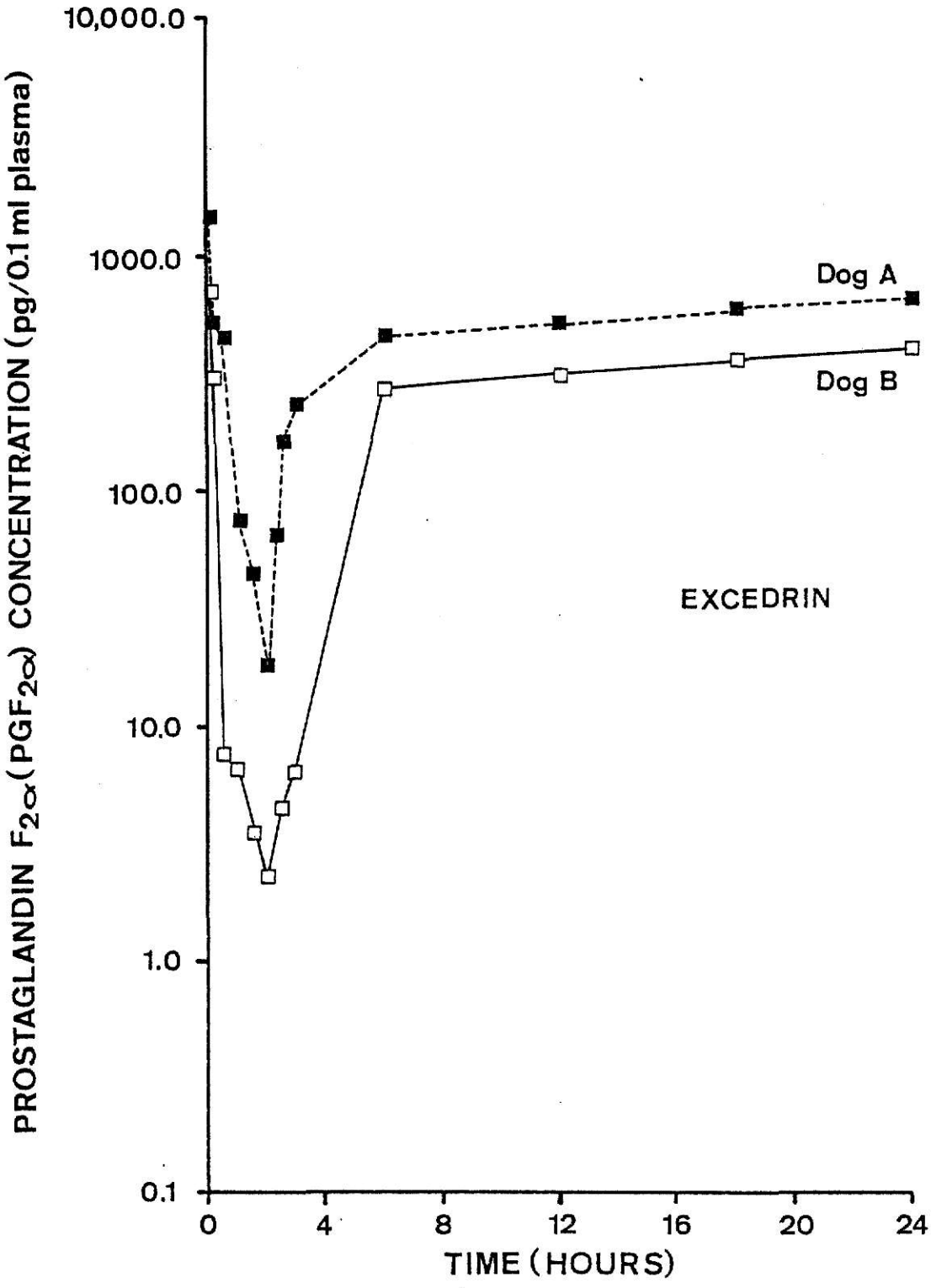
10,000.0
1000.0
100.0
10.0
1.0
0.1
PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$) CONCENTRATION (pg/0.1 ml plasma)
Dog A
Dog B
EXCEDRIN
0
4
8
12
16
20
24
TIME (HOURS)

Figure 2. Prostaglandin-$F_{2\alpha}$ concentrations in blood plasma of dog A (- - -) and dog B (——) after a single dose of 30 mg salicylic acid/kg of body weight as Ascriptin

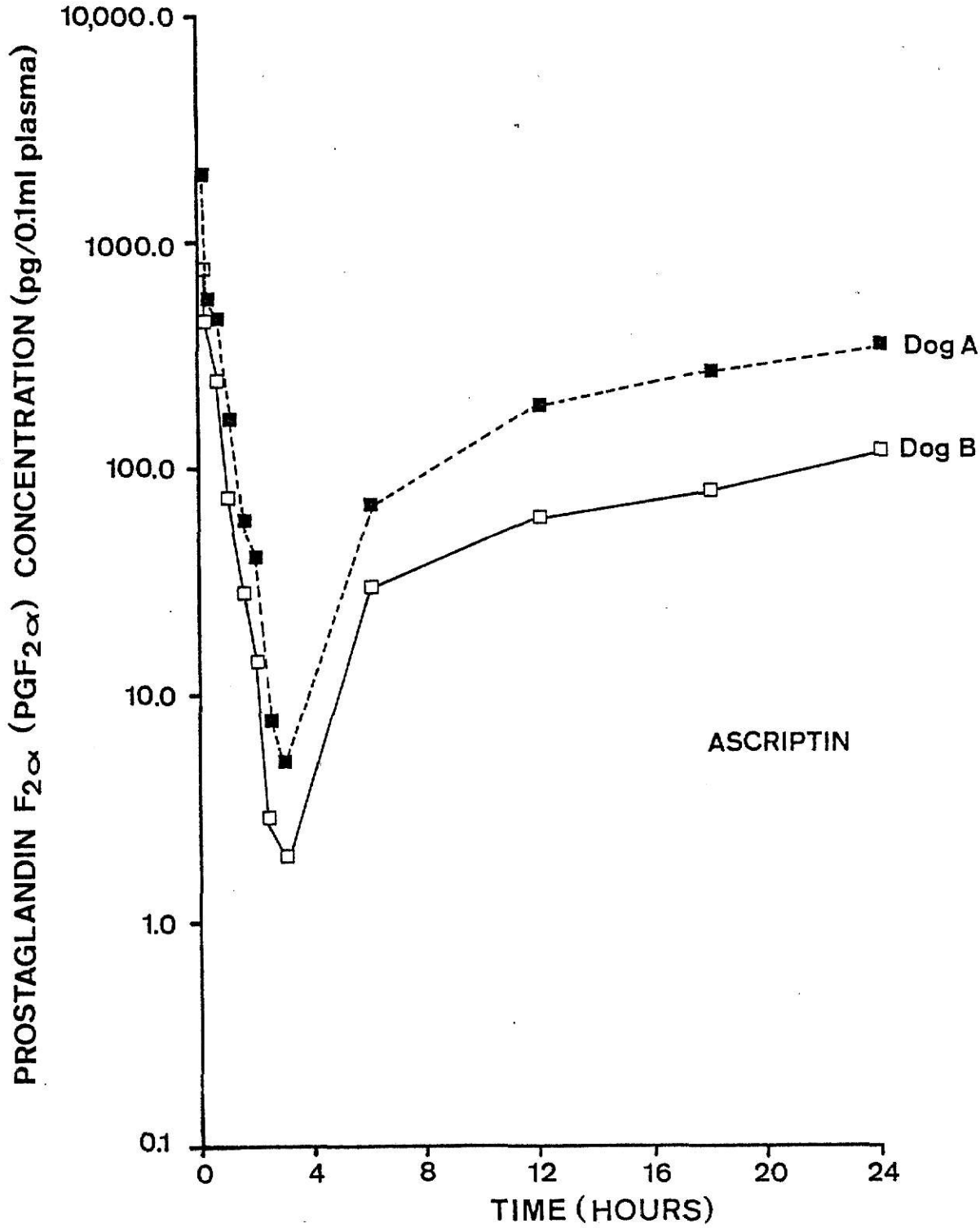
10,000.0
1000.0
100.0
10.0
1.0
0.1
PROSTAGLANDIN F2α (PGF2α) CONCENTRATION (pg/0.1ml plasma)
0
4
8
12
16
20
24
TIME (HOURS)
Dog A
Dog B
ASCRIPTIN

Figure 3. Relationship of percent free prostaglandin-$F_{2\alpha}$ to prostaglandin-$F_{2\alpha}$ concentrations in blood plasma of dog A (– – –) and dog B (———) after a single dose of 30 mg salicylic acid/kg of body weight as Excedrin

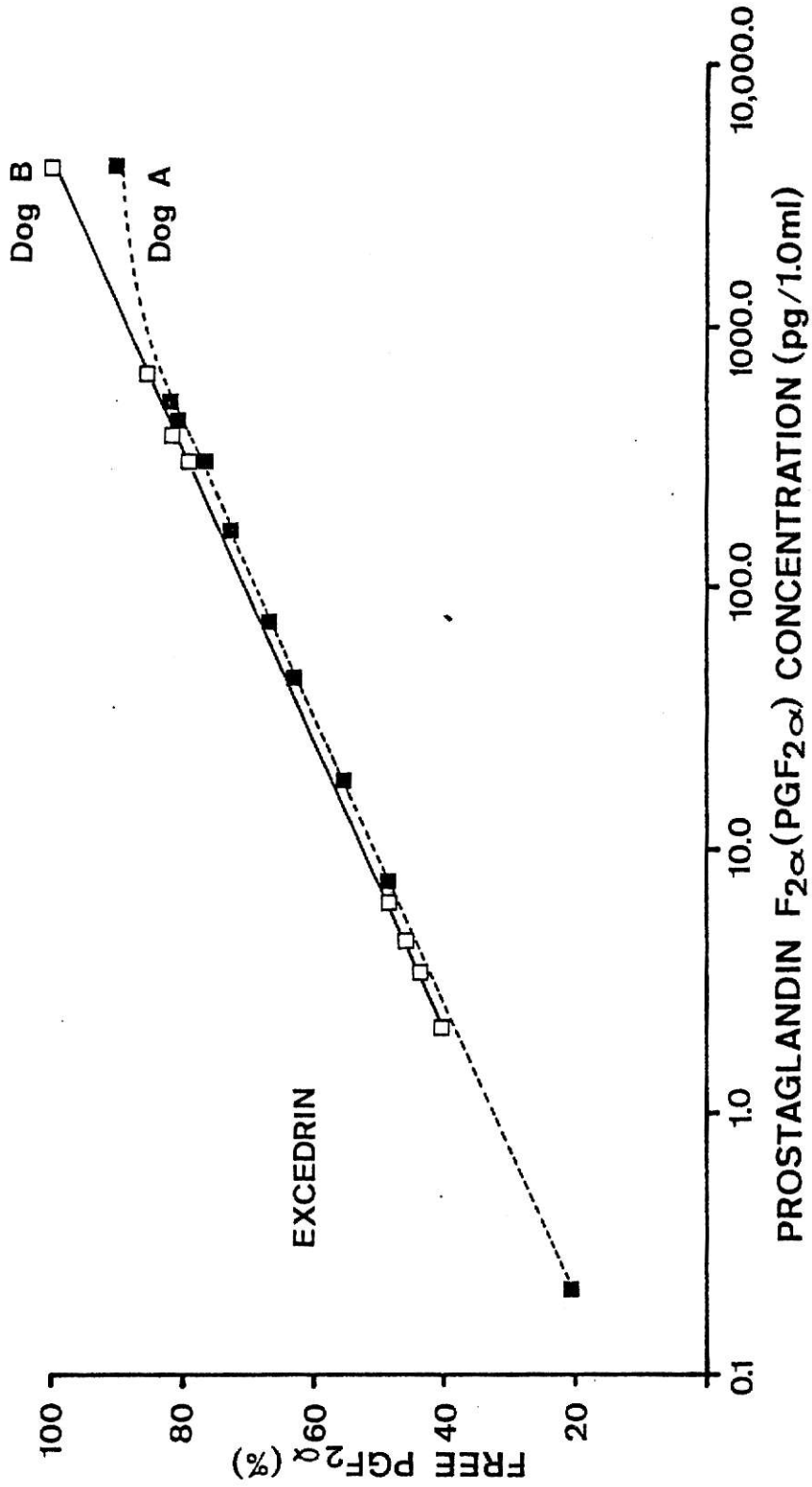
Dog B
Dog A
EXCEDRIN
FREE PGF2α (%)
100
80
60
40
20
0.1
1.0
10.0
100.0
1000.0
10,000.0
PROSTAGLANDIN F2α(PGF2α) CONCENTRATION (pg/1.0ml)

Figure 4. Relationship of percent free prostaglandin- $F_{2\alpha}$ to prostaglandin-$F_{2\alpha}$ concentrations in blood plasma of dog A (- - -) and dog B (———) after a single dose of 30 mg salicylic acid/kg of body weight as Ascriptin

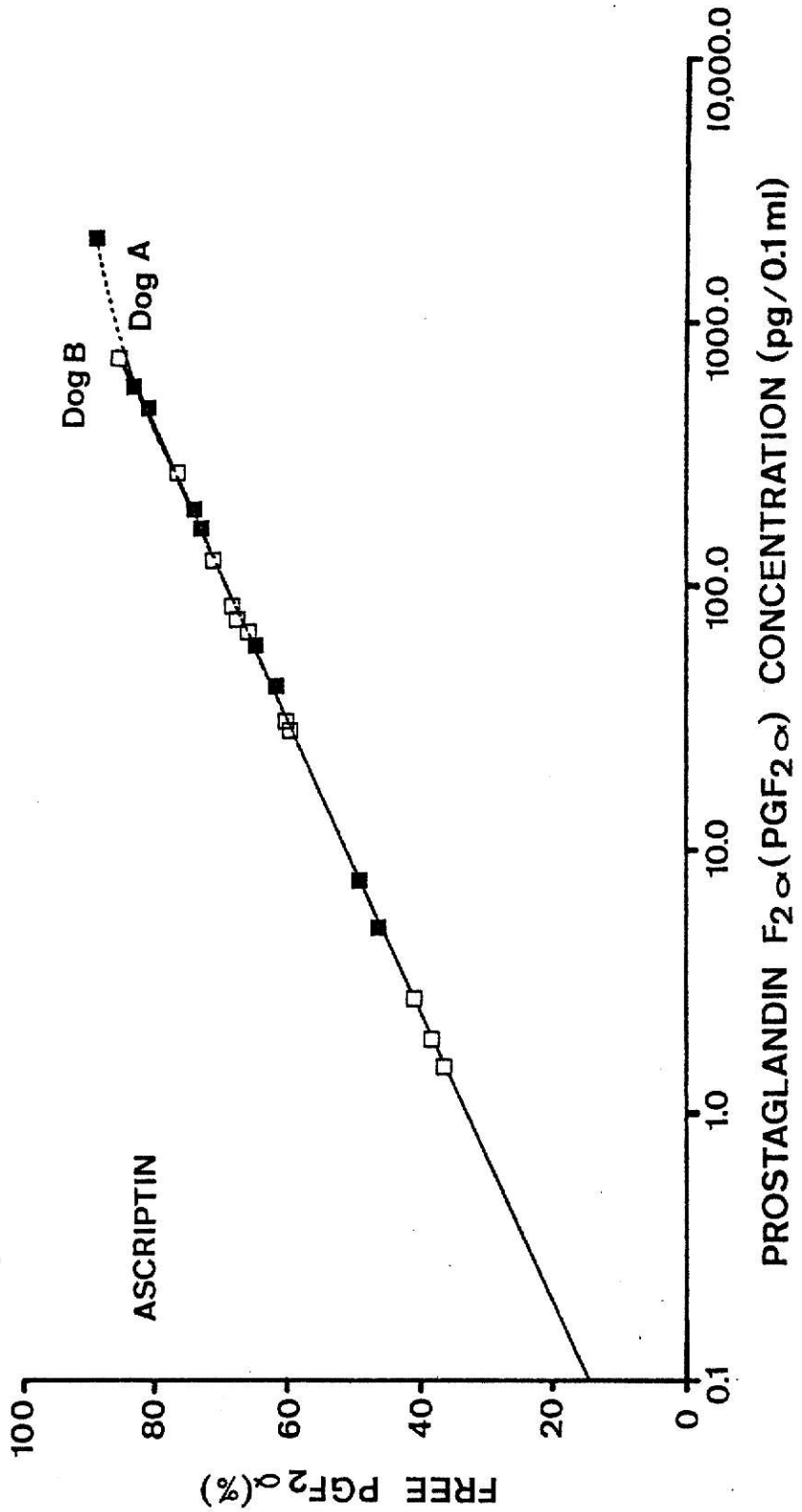
FREE PGF2α(%)
100
80
60
40
20
0
ASCRIPTIN
Dog B
Dog A
0.1
1.0
10.0
100.0
1000.0
10,000.0
PROSTAGLANDIN F2α(PGF2α) CONCENTRATION (pg/0.1ml)

Table 1. Prostaglandin ($PGF_{2\alpha}$) levels in the plasma of two dogs after oral administration of 30 mg of salicylic acid/kg of body weight as Excedrin

Time after dosing (hrs)	Dog A		Dog B	
	$PGF_{2\alpha}$ (g/0.1ml)	Free $PGF_{2\alpha}$ (%)	$PGF_{2\alpha}$ (g/0.1ml)	Free $PGF_{2\alpha}$ (%)
0.0	1400	89.8	700.0	84.9
0.25	500	82.2	300.0	78.3
0.5	450	81.2	7.6	49.0
1.0	74	67.2	6.4	48.4
1.5	45	63.0	3.5	43.2
2.0	18	56.1	2.3	39.9
2.5	160	72.5	4.5	44.7
3.0	230	76.3	6.4	47.8
6.0	450	81.3	270.0	76.8
12.0	501	82.6	320.0	78.8
18.0	600	82.9	350.0	80.0
24.0	660	83.7	400.0	81.0

Table 2. Prostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$) levels in the plasma of two dogs after oral administration of 30 mg of salicylic acid/kg of body weight as Ascriptin

Time after dosing (hrs)	Dog A		Dog B	
	$PGF_{2\alpha}$ (pg/0.1ml)	Free $PGF_{2\alpha}$ (%)	$PGF_{2\alpha}$ (pg/0.1ml)	Free $PGF_{2\alpha}$ (%)
0.0	1200.0	89.1	760.0	85.0
0.25	560.0	82.6	440.0	80.9
0.5	450.0	81.0	240.0	76.1
1.0	160.0	72.8	74.0	67.0
1.5	59.0	64.8	28.0	58.8
2.0	40.0	61.8	14.0	36.2
2.5	7.6	48.9	2.7	41.1
3.0	5.0	46.3	1.9	38.3
6.0	29.0	58.0	30.0	59.8
12.0	180.0	74.0	64.0	66.0
18.0	260.0	76.7	80.0	68.3
24.0	340.0	78.5	121.0	71.0

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APPENDICES

THIS BOOK
CONTAINS
NUMEROUS PAGES
WITH THE ORIGINAL
PRINTING BEING
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DIFFERENTLY FROM
THE TOP OF THE
PAGE TO THE
BOTTOM.

THIS IS AS RECEIVED
FROM THE
CUSTOMER.

A LITERATURE REVIEW OF SALICYLATE COMPOUNDS (O-HYDROXY BENZOIC ACID), $C_7H_6O_3$.

Physical and chemical properties

Salicylic acid occurs as white crystals, usually in fine needles or fluffy crystalline powder. It has a melting point between 158-161 C, and a solubility of 1 g in 460 ml of water[1]. The salicylates used in medicine include the free acid, its sodium salts, its methyl, phenyl, acetylamino and acetyl esters in which substituent groups replace the hydrogen (H) of the hydroxyl group (OH) or the OH of the carboxyl group in the parent molecule, and other derivatives.

The presence of the OH group in the ortho position is esse essential for activity; the parent compound (benzoic acid) is only slightly active. Salicylic acid and methyl salicylate are strong irritant. Methyl salicylate is a volatile oil[2].

Acetyl salicylic acid (Aspirin, $C_9H_8O_4$) exists as white needle shaped crystals or as a crystalline powder. It is odorless, stable in dry air, and has a melting poit of 135 C. It is soluble in water (1 g/300 ml at 25 C), and various organic solvents, such as alcohol, chloroform and ether. Aspirin is incompatible with acetophenetidin, antipyrine, aminopurine, phenol, phenylsalicylate and methenamine. In moist air, aspirin is gradually hydrolysed into salicylic and acetic acids. Aspirin has a molecular weight of 180.16 and its K at 25 C is 3.27×10^{-4},[1].

Sources and exposure

The salicylates and their derivatives were obtained from the

bark of the willow tree which fluorishes along river banks. This willow bark contains salicin, a glycoside of salicylic acid. Acetyl salicylic acid (aspirin), the most widely used of all drugs, was first introduced into medicine in 1876 by Strickler and Reiss[2]. Aspirin is prepared from direct acetylation of salicylic acid, purified by recrystallization from benzene or other non-aqueous solvents[1].

Aspirin and salts of salicylic acid are used as antipyretics, and analgesics in a variety of diseases, such as headache, fevers, muscular aches and pains. Aspirin is used in gout, acute rheumatic fever and in treating rheumatoid arthritis as an anti-inflammation on the synovitis. Aspirin is also used to treat osteoarthritis and ankylosing-spondilitis. Salicylic acid is applied locally in excessive sweating (2-4% in talc)[3,4]. It is also used in various skin preparations to soften or partially dissolve the epidermis due to its kerctalytic action. Methyl salicylate is used topically as a rubifacient. Phenylsalicylate goes through the stomach unchanged and is used to coat gelatin capsules to prevent their disintegration in the stomach[3]. Recently, aspirin has been found to have antithrombotic properties and may prevent cerebrovascular thromboembolism and myocardial infarction[5].

About 15,000 tons of aspirin are produced and supposedly consumed each year. Aspirin is incorporated into at least 400 different drug preparations.

Absorption, tissue distribution, biotransformation and excretion

Most salicylates are administered orally and therefore are

absorbed well and rapidly from the stomach and upper small intestine. Only a small amount is absorbed in the lower intestinal tract. Aspirin absorbed in unionized form is acidic and lipid soluble. The rate of excretion is therefore lower in the acidic environment of the stomach[2,6]. The peak blood level is reached in 2 hrs and varies depending on the dose of aspirin given[4]. The rate of absorption of aspirin tablets depend on the rate of dissolution[7,8]. Aspirin circulates in the blood bound to plasma protein; little is bound to the erythrocytes. Aspirin is distributed into all tissues and even crosses the placenta barrier to the fetus. It is found in highest concentrations in the liver and the kidney. Only small quantities are found in the brain[2].

Davis and Westfall[9] studied the biotransformation and excretion of salicylates. The found that the rate of salicylate disappearance from plasma was related to urinary pH and a species' ability to form metabolites which are more polar than the parent compound. These metabolites include salicyl glucoronides, gentosate (gentisic acid) and salicylurate. Aspirin was bound more strongly to human serum albumin (HSA) than was salicylic acid[10]. Salicylic acid was found to significantly displace phenylbutazone from its binding sites[11].

Analytical Methods

Because of the high affinity salicylates have for plasma protein, many methods have been developed for analysing salicylate concentration in the blood. Trinder[12] determined salicylate in biological fluids using ferric nitrate, mercuric

chloride and hydrochloric acid, which gave a purple color in the presence of salicylic acid. Using both single and double extractiom methods, whole blood or any fraction of blood containing 0-50 mg% salicylic acid can be extracted and read quantatively using ultraviolet spectrophotometer[13]. MacDonald[14] used a modification of Trinder's method in rapid micro and ultramicro procedures. The micro and ultramicro methods gave linear calibration curves of 150 mg salicylate per 100 ml. The phosphorescence of salicylate was measured to avoid interference with other drugs like sulfa and phenacetin by choosing a specific excitation wavelength[15]. Potter and Guy[16] used sephadex gel filtration in conjunction with fluorometric analysis to eliminate large molecular weight compounds present in the plasma with salicylate. Other methods for separating large molecular weight compounds from salicylate include solvent extraction[17] and protein precipitation[12]. Simultaneous determination of aspirin, salicylic acid and salicylamide in plasma using gas liquid chromatography has been reported[18]. Miles and Schenk[19], during fluorometric determination of aspirin in solution containing salicylic acid, found that aspirin also fluoresces but not as much as salicylic acid. Aspirin fluoresces very weakly in chloroform, but the presence of 1% acetic acid, chloroacetic acid or dichloroacetic acid greatly enhances the emission in direct proportion to the strength of these acids[20]. Other methods of determining aspirin and salicylic acid in plasma include fluorometry[16,21], colorimetry, and high pressure liquid chromatography[22,23,24].

Toxicity and Mode of Action

Aspirin, one of the most widely used and safest drugs available, still has some major toxic effects when taken as an overdose or when given to patients who are sensitive to it (i.e., patients with peptic ulcer, alcohol gastritis and esophageal varices). Aspirin is more toxic to the gastric mucosa of man and animals than is salicylic acid[25,26]. Chapman and Duggan[27] suggested that aspirin ingestion often causes peptic ulcer. Aspirin taken in doses of 1-3 g/day will induce occult gastrointestinal bleeding in about 70% of normal subjects; the fetal blood loss of 5 ml/day is five times more than normal[5]. Aspirin intoxication can also produce respiratory alkalosis and metabolic acidosis, either alone or simultaneously. The alkalosis produces a decrease in ionized calcium and tetany[28]. Rothschild[29] suggested that the gastrointestinal bleeding caused by aspirin may result from local rather than systemic toxicity, since aspirin is said to alter the gastric membrane to allow back diffusion of hydrogen ion; this effect depends on the presence of gastric acid (HCl). Other toxic manifestations of aspirin include hyperglycemia or hypoglycemia through the alterations of glucose metabolism, central nervous system (CNS) dysfunction (seizures, coma, cerebral edema), sudden cardiorespiratory arrest (or congestive heart failure), and pulmonary edema.

Aspirin is converted to salicylic acid and acetic acid by the enzyme aspirin esterase[30]. Desbaillets and Masters[31] suggested that differences in relative activity of aspirin

esterase in plasma may account for the sex differences in the distribution of gastric ulcer. Aspirin and the other salicylate compounds have several ways of exerting their actions, and these are the bases of their use as analgesics, antipyretics, anti-inflammatory and antirheumatoid drugs. They affect basic reactions of cellular metabolism. Menguy, Desbaillets and Masters[31] reported a significant decrease in ATP and increases in ADP and AMP levels in the rat gastric mucosa on treatment with aspirin. They postulated that gastric mucosal damage from aspirin might result from cell death due to lack of cellular energy. Mangla, Kim and Rubulis[32], studying aspirin induced gastric mucosal injury in rats, found that aspirin stimulated gastric mucosal adenyl cyclase actvity within 5 minutes of oral administration in vitro and in vivo. Aspirin reduced the energy rich phosphate bonds (ATP, ADP and AMP) more in the gastro-pyloric mucosa area than in the corpus mucosa of mini-pigs[33]. Aspirin uncoupled oxidative phosphorylation by increasing respiration rate (i.e., stimulate oxygen consumption of tissue mitochondria), decreasing respiratory control ratios, and decreasing phosphate/oxygen ratios in vitro. Aspirin causes ultrastructural changes in the liver when used for the treatment of rheumatic fever[34]. This hepatotoxicity of aspirin causes an increase in serum transaminases (both serum glutamic oxaloacetic, SGOT, and serum glutamic pyruvic, SGPT) with other liver enzymes like aldolase and lactic dehydrogenase also decreasing[35]. During inflammation[36], aspirin has been found to inhibit the migration of the two main type of cells involved in acute inflammation,

namely polymorphs and mononuclears. Warne and West[37] reported the inhibition of leucocyte migration by aspirin when edema was induced by subcutaneous injection of carrageenan. Aspirin suppressed erythropoiesis and may produce pseudo-anemia with increased plasma volume, such as seen in peripheral vasodilation[38]. Its effect on the metabolism of pyruvate kinase defficient erythrocytes causes hemolysis[39].

Aspirin affects the synthesis and release of prostaglandins[40] which act as mediators of pain, fever and inflammation. The many therapeutic effects of aspirin may be explained by its inhibition of prostaglandin synthetase or prostaglandin cyclo oxygenase[41]. It has been found that aspirin, but not sodium salicylate, significantly reduced potentiation of paw edema after concurrent administration of carrageenan and arachidonic acid, precursoe of prostaglandin[42,43,44]. Inhibition of prostaglandin synthetase by aspirin is due to acetylation of the enzyme's NH_2-terminal serine[45]. Aspirin desensitizes fructose-1,6-bisphosphotase to AMP inhibition, reduces the enzyme's affinity for substrate and the enzyme's sensitivity to high s substrate inhibition[46]. This is because aspirin competes with dehydrogenases and interacts at the allosteric AMP sites of fructose-1,6-bisphosphotase (fru-P_2ase)[47].

Aspirin reduces renal blood flow and urinary sodium excretion in dogs treated with frusemide to increase the concentration of systemic and local vasoconstricting and vasodilating hormones[48]. Lesions induced by aspirin when given orally, intraperitoneally or intraduodenally, are significantly inhibited by the amino acid, L-glutamine[49].

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INDIVIDUAL EXPERIMENTAL AND ANIMAL DATA

BIOAVAILABILITY STUDIES

Appendix A-1. Fluorescence and salicylate concentrations from Parke Davis brand of aspirin (325 mg) dissolved in artificial gastric juice

Time	Fluorescence								SA conc.
(hrs)	1	2	3	4	5	6	Mean	SD	(mg/250ml)
0.0	1.20	1.31	1.30	1.30	1.30	1.30	1.28	0.04	0.03
0.5	1.49	2.01	1.44	1.50	1.47	1.47	1.58	0.02	0.63
1.0	1.76	2.67	1.69	1.89	1.76	1.89	1.95	0.36	1.38
1.5	1.99	2.21	2.18	2.18	2.50	3.34	2.44	0.47	2.45
2.0	2.57	3.80	2.38	2.69	2.69	2.56	2.80	0.51	3.25
2.5	3.01	4.33	2.75	3.23	3.01	2.94	3.25	0.56	5.13
3.0	3.42	4.55	3.07	4.55	3.69	3.41	3.63	0.50	5.95
3.5	3.82	4.80	3.41	3.41	4.11	3.71	3.97	0.47	6.25
4.0	4.10	4.84	3.61	4.30	4.84	3.99	4.17	0.40	6.50
4.5	4.27	4.82	3.87	4.82	4.58	4.16	4.34	0.33	7.00
5.0	4.49	5.00	5.00	4.10	4.62	4.43	4.53	0.29	7.25
5.5	4.56	5.17	4.27	4.56	4.87	4.66	4.71	0.30	7.28
6.0	4.71	5.05	4.52	4.72	5.04	4.69	4.80	0.21	7.88
6.5	4.81	5.03	4.73	5.07	5.03	4.88	4.90	0.13	8.00
7.0	4.95	5.19	5.19	4.80	5.14	4.84	4.98	0.16	8.25
7.5	5.08	5.33	4.94	5.33	5.26	5.12	5.15	0.14	8.50
8.0	5.09	5.26	5.26	5.24	5.26	5.05	5.18	0.09	5.63
8.5	5.06	5.16	5.06	4.97	5.18	4.96	5.08	0.08	5.75

SD = Standard deviation SA = Salicylic acid

Appendix A-2. Fluorescence and salicylic concentrations from Bufferin brand of aspirin (325 mg) dissolved in artificial gastric juice

Time	Fluorescence								SA conc.
(hrs)	1	2	3	4	5	6	Mean	SD	(mg/250ml)
0.0	1.21	1.21	1.27	1.26	1.25	1.24	1.24	0.02	0.03
0.5	3.72	3.78	3.73	3.76	3.48	3.68	3.69	0.10	5.25
1.0	4.22	4.13	4.20	4.14	3.83	4.01	4.09	0.13	6.13
1.5	4.68	4.53	4.32	4.34	4.01	4.24	4.35	0.21	6.50
2.0	4.81	4.70	4.49	4.59	4.25	4.42	4.54	0.18	7.00
2.5	5.10	4.91	4.63	4.92	4.53	4.77	4.81	0.19	7.50
3.0	5.50	5.23	4.97	5.13	4.78	4.93	5.09	0.23	8.25
3.5	5.35	5.35	4.95	5.43	4.98	5.14	5.20	0.19	8.50
4.0	5.49	5.47	5.20	5.49	5.26	5.20	5.35	0.33	8.88
4.5	5.77	5.62	5.49	5.73	5.21	5.52	5.60	0.19	9.25
5.0	5.55	5.74	5.18	5.75	5.49	5.64	5.56	0.19	9.25
5.5	5.92	5.78	5.32	5.85	5.62	5.73	5.70	0.20	9.63
6.0	6.01	6.04	5.66	5.87	5.75	5.39	5.79	0.22	9.75
6.5	6.00	5.97	5.70	5.91	5,53	5.83	5.82	0.16	9.88
7.0	5.83	5.99	5.66	5.92	5.78	5.64	5.80	0.13	9.75
7.5	5.79	5.87	5.66	5.85	5.79	5.81	5.80	0.07	9.75

SD = Standard deviation

SA = Salicylic acid

Appendix A-3. Fluorescence and salicylate concentrations from Excedrin brand of aspirin (325 mg) dissolved in artificial gastric juice

Time	Fluorescence								SA conc.
(hrs)	1	2	3	4	5	6	Mean	SD	(mg/250ml)
0.0	1.28	1.23	1.24	1.25	1.27	1.25	1.25	0.02	0.03
0.5	7.83	7.07	6.89	7.18	6.88	7.90	7.29	0.42	13.00
1.0	8.75	8.32	7.97	8.16	7.93	8.20	8.22	0.27	14.88
1.5	8.80	8.80	8.34	8.38	8.14	8.77	8.54	0.26	15.63
2.0	9.65	8.90	8.61	8.71	8.65	8.80	8.89	0.35	16.38
2.5	8.86	8.85	8.58	8.56	8.47	8.71	8.67	0.15	16.00
3.0	8.50	8.48	8.72	8.45	8.30	8.56	8.50	0.13	15.50
3.5	8.04	8.52	8.45	8.43	8.56	8.37	8.38	0.17	15.25
4.0	8.41	8.45	8.50	8.24	8.50	8.20	8.38	0.12	15.25
4.5	8.20	8.27	8.55	8.30	8.23	8.11	8.28	0.14	15.13
5.0	8.13	8.29	7.83	8.13	7.87	7.82	8.02	0.18	14.50

SD = Standard deviation

SA = Salicylic acid

Appendix A-4. Fluorescence and salicylate concentrations from Arthritis Strength Formula brand of aspirin (325 mg) dissolved in artificial gastric juice

Time	Fluorescence								SA conc.
(hrs)	1	2	3	4	5	6	Mean	SD	(mg/250ml)
0.0	1.23	1.25	1.25	1.27	1.27	1.28	1.26	0.67	0.03
0.5	2.31	2.06	2.92	2.08	1.96	2.61	2.32	0.34	2.25
1.0	3.01	2.65	3.72	2.82	2.74	3.54	3.08	0.40	4.00
1.5	3.50	3.26	4.16	3.45	3.28	4.25	3.65	0.40	5.25
2.0	4.90	3.81	4.45	3.95	3.68	4.78	4.13	0.55	6.00
2.5	4.49	4.26	5.01	4.44	4.20	5.10	4.58	0.35	7.25
3.0	4.59	4.52	5.15	4.68	4.43	4.99	4.77	0.32	7.50
3.5	4.69	4.70	5.36	4.91	4.72	5.26	4.94	0.27	7.75
4.0	4.73	4.80	5.35	4.94	4.74	5.21	4.96	0.24	7.88
4.5	5.00	4.95	5.40	4.98	4.84	5.41	5.10	0.22	8.25
5.0	5.11	4.87	5.53	5.14	4.95	5.40	5.15	0.21	8.38
5.5	5.08	5.11	5.66	5.22	5.08	5.60	5.26	0.31	8.50
6.0	5.05	5.17	5.62	5.32	4.97	5.63	5.26	0.27	8.63
6.5	5.36	5.40	5.62	5.25	5.07	5.59	5.37	0.17	8.88
7.0	5.20	5.26	5.50	5.18	4.97	5.62	5.29	0.21	8.63
7.5	5.08	5.15	5.60	5.25	5.14	5.40	5.27	0.18	8.63
8.0	5.27	5.20	5.57	5.32	5.20	5.78	5.38	0.24	8.88

SD = Standard deviation

SA = Salicylic acid

Appendix A-5. Fluorescence and salicylate concentrations from Excedrin P.M. brand of aspirin (325 mg) dissolved in artificial gastric juice

Time	Fluorescence								SA conc.
(hrs)	1	2	3	4	5	6	Mean	SD	(mg/250ml)
0.0	1.24	1.32	1.24	1.27	1.26	1.25	1.26	0.03	0.03
0.5	4.54	3.97	3.52	3.79	3.20	3.51	3.76	0.43	5.38
1.0	3.74	3.27	3.58	3.37	3.37	3.74	3.52	0.19	4.75
1.5	3.37	3.46	3.73	3.47	3.46	3.82	3.55	0.16	4.88
2.0	3.26	3.40	3.84	3.54	3.47	3.71	3.54	0.19	4.88
2.5	3.20	3.50	4.03	3.72	3.63	3.88	3.66	0.27	5.00
3.0	3.38	3.77	3.76	3.73	3.57	3.71	3.65	0.14	5.13
3.5	3.41	3.75	3.78	3.79	3.70	3.85	3.71	0.14	5.25
4.0	3.60	3.84	3.76	3.83	3.68	3.83	3.76	0.09	5.25
4.5	3.53	3.71	3.73	3.90	3.72	3.73	3.70	0.08	5.25
5.0	3.52	3.81	3.81	3.88	3.78	3.88	3.78	0.12	5.50
5.5	3.52	3.71	3.76	3.76	3.77	3.75	3.71	0.09	5.25
6.0	3.68	3.81	3.82	3.84	3.90	3.87	3.82	0.07	5.63
6.5	3.66	3.78	3.67	3.70	3.78	3.81	3.73	0.06	5.25
7.0	3.62	3.88	3.76	3.85	3.95	3.83	3.82	0.10	5.63
7.5	3.73	3.80	3.94	3.90	3.84	3.80	3.84	0.07	5.63
8.0	3.80	3.83	3.83	3.72	3.77	3.82	3.80	0.04	5.50
8.5	3.88	3.87	3.99	3.90	3.89	3.83	3.89	0.05	5.75
9.0	3.80	3.93	3.88	3.88	3.85	3.80	3.86	0.05	5.75
9.5	3.67	3.80	3.69	3.80	3.92	4.00	3.80	0.12	5.50
10.0	3.66	3.77	3.63	3.77	3.87	3.90	3.77	0.10	5.38
10.5	3.65	3.76	3.60	3.76	3.87	3.86	3.75	0.10	5.38

SD = Standard deviation SA = Salicylic acid

Appendix A-6. Fluorescence and salicylate concentration from Arthritis Strength Bufferin brand of aspirin (325 mg) dissolved in gastric juice

Time	Fluorescence								SA conc.
(hrs)	1	2	3	4	5	6	Mean	SD	(mg/250ml)
0.0	1.30	1.32	1.32	1.31	1.31	1.29	1.31	0.01	0.03
0.5	3.47	3.96	3.20	3.87	4.20	4.48	3.86	0.43	5.63
1.0	4.04	4.90	4.34	4.52	5.04	5.05	4.55	0.33	7.00
1.5	4.40	5.18	4.82	5.05	5.32	5.46	5.04	0.35	8.00
2.0	4.60	5.65	5.28	5.31	5.54	5.63	5.34	0.36	8.63
2.5	4.61	5.74	5.46	5.42	5.65	5.98	5.48	0.43	9.25
3.0	4.91	5.95	5.83	5.64	6.18	6.11	5.77	0.42	9.75
3.5	5.37	6.45	6.30	5.96	5.89	6.10	6.01	0.79	10.25
4.0	5.47	5.97	6.09	5.88	6.12	6.30	5.97	0.26	10.25
4.5	5.53	5.90	6.16	5.85	6.00	5.93	5.90	0.19	10.00
5.0	6.86	6.30	6.36	6.47	6.42	6.68	6.52	0.19	10.00
5.5	6.38	6.68	6.00	6.50	6.35	6.52	6.41	0.21	11.25
6.0	6.12	6.48	6.16	6.37	6.57	6.81	6.49	0.21	11.25
6.5	6.15	6.60	6.60	6.61	6.67	6.98	6.60	0.24	11.50
7.0	6.43	6.61	6.81	6.51	6.62	6.95	6.66	0.18	11.75
7.5	6.52	6.85	6.92	6.70	6.80	7.34	6.86	0.25	12.25
8.0	6.44	6.70	6.87	6.70	6.67	7.10	6.75	0.20	11.75
8.5	6.50	6.98	7.18	6.93	6.70	7.05	6.89	0.22	12.25
9.0	6.60	6.94	7.03	6.83	6.66	7.13	6.87	0.18	11.75
9.5	6.47	7.07	7.02	6.90	6.68	7.20	6.89	0.25	12.25
10.0	6.67	7.01	6.87	6.62	6.68	7.16	6.83	0.20	12.00
10.5	6.66	6.99	7.03	6.90	6.78	7.30	6.88	0.24	12.25

SD = Standard deviation SA = Salicylic acid

Appendix A-7. Fluorescence and salicylate concentrations from Purity brand of aspirin (325 mg) dissolved in artificial gastric juice

Time	Fluorescence								SA conc.
(hrs)	1	2	3	4	5	6	Mean	SD	(mg/250ml)
0.0	1.25	1.24	1.23	1.24	1.24	1.22	1.24	0.01	0.03
0.5	1.31	1.25	1.34	1.26	1.32	1.34	1.30	0.03	0.50
1.0	1.59	1.40	1.77	1.51	1.60	1.81	1.61	0.14	0.73
1.5	1.92	1.67	2.37	2.00	2.08	2.41	2.08	0.26	1.88
2.0	2.41	2.00	2.79	2.46	2.68	3.00	2.56	0.32	2.63
2.5	2.59	2.38	3.49	2.97	3.09	3.56	2.85	0.40	3.25
3.0	3.10	2.81	3.80	3.36	3.47	4.06	3.43	0.42	4.63
3.5	3.52	3.08	4.22	3.79	3.80	4.23	3.77	0.40	5.50
4.0	3.74	3.35	4.35	4.02	4.04	4.35	3.98	0.35	5.95
4.5	4.00	3.61	4.51	4.26	4.42	4.68	4.25	0.35	6.25
5.0	4.31	3.87	4.65	4.51	4.45	4.77	4.43	0.29	6.75
5.5	4.39	4.12	4.59	4.35	4.77	4.78	4.50	0.24	7.00
6.0	4.26	4.26	4.71	4.54	4.52	4.69	4.50	0.18	7.00
6.5	4.53	4.36	4.58	4.68	4.84	5.03	4.67	0.22	7.50
7.0	4.75	4.55	4.89	4.68	4.87	4.92	4.78	0.13	7.50
7.5	4.61	4.60	4.83	4.60	4.68	4.75	4.68	0.09	7.38
8.0	4.63	4.51	4.29	5.10	4.95	5.06	4.76	0.30	7.50
8.5	5.02	4.83	5.06	4.97	4.80	4.81	4.91	0.10	7.75
9.0	4.76	4.92	5.03	4.98	4.83	4.92	4.91	0.09	7.75
9.5	5.03	4.68	5.18	5.14	5.03	5.01	5.01	0.16	8.00

Apendix A-7 cont'd.

Time	Fluorescence								SA conc.
(hrs)	1	2	3	4	5	6	Mean	SD	(mg/250ml)
10.0	4.93	4.98	5.01	5.12	5.03	4.99	5.04	0.08	8.00
10.5	4.94	5.04	5.16	4.86	4.99	5.08	5.01	0.10	8.00
11.0	4.94	4.97	4.95	4.76	5.00	4.92	4.92	0.08	7.75

SD = Standard deviation

SA = Salicylic acid

Appendix A-9. Fluorescence and salicylate concontrations from Ascriptin brand of aspirin (325 mg) dissolved in artificial gastric juice

Time	Fluorescence								SA conc.
(hrs)	1	2	3	4	5	6	Mean	SD	(mg/250ml)
0.0	1.22	1.21	1.21	1.21	1.19	1.21	1.21	0.01	0.03
0.5	1.63	1.45	1.53	1.33	1.37	1.63	1.49	0.12	0.75
1.0	2.00	1.87	2.05	1.90	1.71	2.30	1.97	0.18	1.75
1.5	2.47	2.29	2.75	2.68	2.01	3.00	2.53	0.32	3.00
2.0	3.00	2.87	3.55	3.19	2.57	3.82	3.17	0.42	4.25
2.5	3.35	3.13	3.91	3.73	2.81	4.31	3.54	0.50	5.25
3.0	3.57	3.40	4.36	3.91	3.22	4.90	3.99	0.54	6.25
3.5	4.02	3.98	4.99	4.57	3.77	5.53	4.48	0.62	7.25
4.0	4.58	4.31	5.52	4.86	3.92	5.95	4.92	0.62	8.13
4.5	4.75	4.62	4.67	5.28	4.27	6.31	5.16	0.69	8.75
5.0	5.09	4.82	6.07	5.33	4.58	6.52	5.40	0.68	9.13
5.5	5.40	5.10	6.28	5.65	5.09	6.77	5.72	0.62	9.88
6.0	5.69	5.50	6.60	5.90	4.94	6.68	5.89	0.61	10.25
6.5	6.01	5.64	6.65	6.16	5.13	6.76	6.06	0.56	10.50
7.0	5.86	5.99	6.80	6.24	5.27	6.93	6.18	0.56	10.88
7.5	6.12	5.84	6.90	6.30	5.68	7.23	6.35	0.55	11.13
8.0	6.35	6.24	7.23	6.52	5.75	7.14	6.54	0.51	11.63
8.5	6.44	6.11	6.97	6.59	6.10	7.37	6.63	0.45	11.88
9.0	6.62	6.23	7.09	6.55	5.73	7.13	6.56	0.48	11.75
9.5	6.44	6.20	7.01	6.87	6.32	7.35	6.70	0.41	12.25
10.0	6.70	6.25	7.10	6.92	6.13	7.09	6.70	0.40	12.25
10.5	6.53	6.30	6.99	6.48	6.48	7.54	6.72	0.42	12.25

SD = Standard deviation SA = Salicylic acid

Appendix A-8. Fluorescence and salicylate concentrations from Squibb brand of aspirin (325 mg) dissolved in artificial gastric juice

Time	Fluorescence								SA conc.
(hrs)	1	2	3	4	5	6	Mean	SD	(mg/250ml)
0.0	1.26	1.28	1.29	1.20	1.27	1.26	1.26	0.03	0.03
0.5	1.45	1.59	1.75	1.57	1.64	1.61	1.60	0.09	0.75
1.0	2.02	2.15	2.47	2.18	2.30	2.30	2.24	0.14	2.00
1.5	2.40	2.71	3.09	2.69	2.72	2.88	2.75	0.21	4.50
2.0	3.01	3.11	3.75	3.21	3.31	3.55	3.32	0.25	5.75
2.5	3.93	3.80	4.02	3.75	3.86	4.17	3.92	0.14	6.25
3.0	4.20	4.09	4.44	4.21	4.08	4.18	4.20	0.12	7.25
3.5	4.49	4.50	4.71	4.45	4.42	4.75	4.55	0.13	7.38
4.0	4.71	4.68	4.85	4.74	4.58	4.77	4.72	0.08	7.50
4.5	4.69	4.75	4.84	4.62	4.74	4.82	4.74	0.07	7.88
5.0	4.73	4.57	4.72	4.60	4.49	4.82	4.66	0.11	7.38
5.5	4.85	4.90	4.81	5.00	4.85	4.95	4.89	0.06	7.50
6.0	4.85	4.75	4.84	4.74	4.65	4.60	4.74	0.09	7.50
6.5	4.93	4.63	4.73	4.74	4.79	5.02	4.81	0.13	7.50
7.0	4.81	4.82	4.72	4.54	4.93	4.80	4.77	0.12	7.50
7.5	4.83	4.81	4.82	4.80	4.68	4.68	4.73	0.12	7.38
8.0	4.54	4.28	5.96	4.74	4.88	4.93	4.89	0.52	7.88
8.5	4.78	4.92	4.96	4.71	4.82	4.76	4.83	0.08	7.50

SD = Standard deviation

SA = Salicylic acid

Appendix A-10. Fluorescence and salicylate concentrations from Bayer brand of aspirin (325 mg) dissolved in artificial gastric juice

Time	Fluorescence								SA conc.
(hrs)	1	2	3	4	5	6	Mean	SD	(mg/250ml)
0.0	1.22	1.20	1.26	1.27	1.23	1.25	1.24	0.02	0.03
0.5	1.37	1.42	1.39	1.28	1.38	1.57	1.40	0.09	0.25
1.0	1.72	1.84	1.97	1.62	1.72	2.28	1.86	0.22	1.25
1.5	2.23	2.49	2.35	1.91	2.17	3.04	2.37	0.35	2.25
2.0	2.70	3.07	2.94	2.37	2.47	3.48	2.83	0.37	3.25
2.5	3.08	3.55	3.25	2.55	3.18	2.83	2.99	0.34	3.75
3.0	3.52	5.10	3.77	3.08	3.53	4.35	3.67	0.45	5.38
3.5	3.86	4.32	3.95	3.38	3.83	4.52	3.92	0.40	5.75
4.0	4.03	4.47	4.07	3.70	3.89	4.76	4.15	0.36	6.25
4.5	4.30	4.62	4.47	3.86	4.27	4.86	4.33	0.37	6.50
5.0	4.41	4.88	4.55	4.24	4.09	5.01	4.56	0.29	7.25
5.5	4.40	4.79	4.62	4.14	4.52	4.85	4.49	0.29	7.13
6.0	4.41	4.62	4.74	4.54	4.50	5.02	4.64	0.19	7.38
6.5	4.79	4.85	4.68	4.45	4.62	5.12	4.68	0.15	7.38
7.0	4.74	4.80	5.02	4.74	4.80	4.97	4.84	0.17	7.50
7.5	4.98	5.04	4.85	4.63	4.50	4.98	4.88	0.14	7.75
8.0	4.61	4.91	4.74	4.50	4.88	5.00	4.71	0.19	7.28
8.5	4.80	4.88	4.77	4.65	4.88	4.94	4.83	0.12	7.50
9.0	5.00	5.00	4.90	4.70	5.20	5.04	4.89	0.11	7.75
9.5	4.82	4.82	4.68	4.53	4.95	4.95	4.85	0.22	7.50

SD = Standard deviation SA = Salicylic acid

Appendix A-11. Fluorescence and salicylate concentrations from Empirin brand of aspirin (325 mg) dissolved in artificial gastric juice

Time	Fluorescence								SA conc.
(hrs)	1	2	3	4	5	6	Mean	SD	(mg/250ml)
0.0	1.21	1.23	1.20	1.19	1.20	1.20	1.21	0.01	0.03
0.5	1.40	1.33	1.25	1.35	1.52	1.27	1.35	0.09	0.25
1.0	1.88	1.80	1.72	1.84	2.18	1.85	1.88	0.14	1.63
1.5	2.48	2.18	2.05	2.14	2.60	3.62	2.51	0.53	2.88
2.0	4.57	3.20	3.78	2.76	3.38	2.94	3.44	0.60	4.88
2.5	3.51	3.21	3.15	3.25	3.97	3.59	3.45	0.28	4.88
3.0	4.02	3.78	3.58	3.71	4.34	3.95	3.90	0.25	6.00
3.5	4.34	4.10	4.02	3.97	4.56	4.26	4.21	0.20	6.50
4.0	4.60	4.32	4.22	4.20	4.67	4.40	4.40	0.18	7.00
4.5	4.73	4.55	4.31	4.31	4.79	4.57	4.54	0.18	7.25
5.0	4.78	4.67	4.77	4.65	5.05	4.80	4.79	0.13	7.75
5.5	4.93	4.84	4.83	4.91	4.96	4.90	4.90	0.05	8.00
6.0	5.01	4.95	5.02	4.89	5.04	5.02	5.00	0.05	8.25
6.5	5.08	5.07	5.02	4.87	5.20	5.22	5.08	0.11	8.50
7.0	5.08	5.14	4.94	4.93	5.01	5.10	5.00	0.12	8.25
7.5	4.91	5.10	5.02	4.96	5.05	4.75	4.95	0.14	8.25
8.0	5.03	5.15	5.02	4.90	4.93	4.66	4.94	0.15	8.00

SD = Standard deviation

SA = Salicylic acid

Appendix A-12. Measured fluorescence and fitrate salicylate concentrations of dogA plasma, after treatment with 30 mg salicylic acid/kg body weight as Excedrin

Time	Fluorescence					SA conc.
(hrs)	1	2	3	Mean*	SD	(mg/ml)
0.0	0.42	0.34	0.31	0.35	0.05	0.001
0.5	6.62	6.61	6.56	6.60	0.03	0.012
1.0	7.08	7.21	7.06	7.12	0.07	0.013
1.5	5.68	5.80	5.69	5.72	0.05	0.011
2.0	10.14	11.23	10.67	10.68	0.04	0.020
2.5	7.10	7.06	7.02	7.06	0.03	0.013
3.0	6.94	6.91	6.86	6.90	0.03	0.013
3.5	5.83	5.85	5.78	5.82	0.03	0.011
4.0	5.81	5.80	5.73	5.78	0.04	0.011
4.5	4.72	4.68	4.65	4.68	0.03	0.009
5.0	4.16	4.20	4.10	4.15	0.04	0.008
5.5	6.27	6.23	6.20	6.23	0.03	0.012
6.0	5.44	5.43	5.43	5.43	0.00	0.010
6.5	3.47	3.48	3.43	3.46	0.02	0.007
12.0	2.05	2.08	2.07	2.07	0.02	0.004
24.0	5.00	5.20	4.81	5.03	0.02	0.006

*Dilution factor of 100

Appendix A-13. Measured fluorescence and concentrate salicylate content of dog A plasma after treatment with 30 mg salicylic acid/kg body weight as Excedrin

Time	Fluorescence					SA conc.
(hrs)	1	2	3	Mean*	SD	(mg/ml)
hrs						mg/ml
0.0	0.16	0.16	0.16	0.16**	0.00	0.001
0.5	17.01	17.03	17.02	17.02**	0.01	0.060
1.0	17.06	17.09	17.12	17.09**	0.02	0.068
1.5	16.79	16.78	16.78	16.78***	0.00	0.125
2.0	13.32	13.32	13.00	13.21	0.15	0.123
2.5	12.64	12.57	12.47	12.56	0.07	0.118
3.0	11.59	11.63	11.57	11.59	0.02	0.108
3.5	16.83	16.73	16.70	16.75	0.06	0.250
4.0	9.16	9.38	9.13	9.22	0.11	0.089
4.5	14.40	14.51	14.14	14.35	0.16	0.135
5.0	16.00	16.12	15.84	15.98	0.11	0.150
5.5	15.25	15.24	15.13	15.21	0.05	0.143
6.0	11.11	11.15	11.12	11.13	0.02	0.103
6.5	11.47	11.46	11.34	11.42	0.06	0.105
12.0	6.08	6.03	6.23	6.11	0.06	0.012
24.0	3.00	3.03	3.04	3.02	0.01	0.009

*Dilution factor of 500 except where otherwise indicated

**Dilution factor of 100

***Dilution factor of 250

Appendix A-14. Measured fluorescence and filtrate salicylate concentrations of dog B plasma after treatment with 30 mg salicylic acid/kg body weight as Excedrin

Time	Fluorescence					SA conc.
(hrs)	1	2	3	Mean*	SD	(mg/ml)
0.0	1.11	1.12	1.10	1.11	0.01	0.002
0.5	6.15	6.24	6.36	6.25	0.82	0.012
1.0	10.60	10.74	10.49	10.61	0.10	0.020
1.5	11.20	11.10	10.92	11.07	0.12	0.021
2.0	15.09	15.07	15.08	15.08	0.01	0.028
2.5	14.24	14.14	13.92	14.10	0.13	0.026
3.0	14.51	14.78	14.96	14.75	0.18	0.028
3.5	12.67	12.70	12.55	12.64	0.06	0.024
4.0	13.95	14.01	13.72	13.89	0.12	0.026
4.5	10.95	10.91	10.75	10.87	0.09	0.020
5.0	8.83	8.76	8.90	8.83	0.06	0.016
5.5	13.16	13.07	12.75	12.99	0.17	0.024
6.0	10.03	10.10	9.95	10.03	0.06	0.019
6.5	7.52	7.30	7.20	7.34	0.13	0.014
12.0	2.05	2.08	2.07	2.07	0.02	0.004
24.0	1.39	1.42	1.37	1.39	0.02	0.002

*Dilution factor of 100

Appendix A-15. Measured fluorescence and concentrate salicylate content of dog B plasma after treatment with 30 mg salicylic acid/kg body weight as Excedrin

Time	Fluorescence					SA conc.
(hrs)	1	2	3	Mean*	SD	(mg/ml)
0.0	0.16	0.14	0.21	0.17	0.03	0.026
0.5	8.75	8.73	8.73	8.78	0.06	0.130
1.0	8.20	8.38	8.26	8.28	0.07	0.155
1.5	9.56	9.69	9.52	9.59	0.07	0.180
2.0	10.15	10.19	10.07	10.14	0.05	0.180
2.5	10.37	10.30	10.31	10.33	0.03	0.190
3.0	8.66	8.77	8.64	8.69	0.05	0.160
3.5	9.12	8.94	8.82	8.96	0.12	0.165
4.0	8.33	8.32	8.31	8.32	0.01	0.155
4.5	8.31	8.31	8.29	8.30	0.01	0.155
5.0	7.88	7.89	7.74	7.84	0.01	0.145
5.5	8.10	8.12	8.07	8.10	0.02	0.150
6.0	7.33	7.45	7.33	7.37	0.06	0.135
6.5	7.30	7.40	7.41	7.37	0.06	0.135
12.0	8.56	8.58	8.56	8.57	0.02	0.080
24.0	5.09	5.00	5.15	5.08	0.05	0.045

*Dilution factor of 500

Appendix A-16. Measured fluorescence and filtrate salicylate concentrations Of dog A plasma, after treatment with 30 mg salicylic acid/kg body weight as Ascriptin

Time	Fluorescence					SA conc.
(hrs)	1	2	3	Mean*	SD	(mg/ml)
0.0	0.28	0.28	0.30	0.29	0.01	0.005
0.5	3.30	3.19	3.20	3.23	0.05	0.006
1.0	3.79	3.80	3.78	3.79	0.01	0.007
1.5	5.23	5.23	5.16	5.21	0.03	0.010
2.0	3.04	3.00	2.95	3.00	0.04	0.007
2.5	6.76	6.72	6.62	6.70	0.06	0.013
3.0	7.36	7.44	7.35	7.38	0.04	0.014
3.5	6.75	6.64	6.69	6.69	0.04	0.013
4.0	3.32	3.32	3.30	3.31	0.01	0.006
4.5	2.66	2.67	2.61	2.65	0.03	0.005
5.0	3.82	3.82	3.77	3.80	0.02	0.007
5.5	4.19	4.15	4.10	4.15	0.04	0.008
6.0	5.02	5.05	4.96	5.01	0.04	0.010
6.5	5.32	5.30	5.28	5.30	0.02	0.011
7.0	2.54	2.52	2.43	2.48	0.04	0.005
7.5	4.23	4.21	4.16	4.20	0.03	0.002
8.0	3.42	3.42	3.43	3.42	0.01	0.006
8.5	3.16	3.16	3.17	3.16	0.01	0.006
9.0	3.87	3.62	3.68	3.72	0.11	0.007
9.5	2.94	3.03	3.13	3.03	0.08	0.006
10.0	2.08	2.07	2.05	2.07	0.01	0.004

Appendix A-16 cont'd.

Time	Fluorescence					SA conc.
(hrs)	1	2	3	Mean*	SD	(mg/ml)
10.5	3.42	3.37	3.35	3.38	0.03	0.006
11.0	2.35	2.30	2.28	2.31	0.03	0.004
11.5	1.83	1.84	1.81	1.83	0.01	0.003
12.0	2.92	2.94	2.94	2.93	0.01	0.005
24.0	2.92	2.90	2.88	2.90	0.01	0.005

*Dilution factor of 100

Appendix A-17. Measured fluorescence and concentrate salicylate content of dog A plasma, after treatment with 30 mg salicylic acid/kg body weight as Ascriptin

Time (hrs)	Fluorescence					SA conc. (mg/ml)
	1	2	3	Mean*	SD	
0.0	0.18	0.18	0.20	0.19	0.01	0.070
0.5	4.81	4.74	4.62	4.72	0.08	0.088
1.0	4.73	4.77	4.64	4.71	0.05	0.088
1.5	6.49	6.28	6.23	6.33	0.11	0.115
2.0	5.31	5.48	5.44	5.41	0.07	0.100
2.5	5.51	5.58	5.50	5.53	0.04	0.100
3.0	4.28	4.38	4.44	4.37	0.07	0.080
3.5	7.35	8.26	9.35	8.32	0.82	0.155
4.0	7.35	6.82	7.01	7.06	0.22	0.130
4.5	5.55	5.48	5.55	5.53	0.03	0.102
5.0	6.22	6.24	6.20	6.22	0.02	0.110
5.5	5.72	5.64	5.65	5.67	0.04	0.105
6.0	4.97	5.07	5.02	5.02	0.04	0.090
6.5	5.65	5.75	5.73	5.71	0.04	0.105
7.0	4.67	4.90	4.86	4.81	0.10	0.090
7.5	5.50	5.69	5.63	5.61	0.08	0.100
8.0	4.95	5.13	5.05	5.04	0.07	0.090
8.5	4.18	4.13	4.15	4.15	0.02	0.075
9.0	5.16	5.25	5.18	5.20	0.04	0.100
9.5	4.43	4.53	4.45	4.47	0.04	0.085
10.0	5.61	5.67	5.71	5.66	0.04	0.105

Appendix A-17 cont'd.

Time	Fluorescence					SA conc.
(hrs)	1	2	3	Mean*	SD	(mg/ml)
10.5	5.90	6.00	5.98	5.96	0.04	0.110
11.0	4.15	4.23	4.20	4.19	0.03	0.075
11.5	5.19	5.29	5.24	5.24	0.04	0.095
12.0	4.39	4.42	4.39	4.40	0.01	0.080
24.0	3.90	4.01	3.84	3.92	0.02	0.055

*Dilution factor of 1000

Appendix A-18. Measured fluorescence and filtrate salicylate concentrations of dog B plasma, after treatment with 30 mg salicylic acid/kg body weight as Ascriptin

Time	Fluorescence					SA conc.
(hrs)	1	2	3	Mean*	SD	(mg/ml)
0.0	0.31	0.32	0.30	0.31	0.01	0.006
0.5	2.00	2.00	1.99	2.00	0.01	0.004
1.0	7.11	7.04	7.01	7.06	0.04	0.013
1.5	8.67	8.74	8.70	8.70	0.03	0.016
2.0	11.31	11.70	11.60	11.54	0.17	0.022
2.5	13.18	13.18	13.23	13.20	0.02	0.026
3.0	8.04	7.93	8.12	8.03	0.08	0.015
3.5	7.86	7.93	7.88	7.89	0.03	0.015
4.0	9.15	9.17	9.14	9.15	0.01	0.017
4.5	8.96	9.08	9.17	9.07	0.09	0.017
5.0	6.47	6.54	6.51	6.51	0.03	0.012
5.5	11.16	11.06	11.05	11.09	0.05	0.021
6.0	7.01	6.92	7.02	6.98	0.05	0.013
6.5	9.13	9.11	8.93	9.06	0.09	0.018
7.0	6.56	6.71	6.57	6.63	0.07	0.013
7.5	6.32	6.33	6.41	6.35	0.04	0.012
8.0	5.85	5.92	5.99	5.92	0.06	0.010
8.5	8.13	8.05	8.06	8.08	0.04	0.015
9.0	5.23	5.29	5.29	5.27	0.03	0.010
9.5	4.66	4.75	4.71	4.71	0.04	0.009
10.0	5.43	5.45	5.34	5.41	0.05	0.010

Appendix A-18 cont'd.

Time (hrs)	Fluorescence					SA conc. (mg/ml)
	1	2	3	Mean*	SD	
						mg/ml
10.5	5.85	5.69	5.63	5.72	0.09	0.015
11.0	6.35	6.36	6.26	6.32	0.05	0.013
11.5	5.58	5.59	5.52	5.56	0.03	0.010
12.0	4.31	4.35	4.34	4.33	0.02	0.008
24.0	8.56	8.58	8.56	8.57	0.02	0.016

*Dilution factor of 100

Appendix A-19. Measured fluorescence and concentrate salicylate content of dog B plasma, after treatment with 30 mg salicylic acid/kg body weight as Ascriptin

Time	Fluorescence					SA conc.
(hrs)	1	2	3	Mean*	SD	(mg/ml)
0.0	6.90	7.20	7.90	7.30	0.05	0.015
0.5	10.26	10.35	10.24	10.28	0.05	0.065
1.0	12.62	12.66	12.37	12.55	0.13	0.110
1.5	12.53	12.55	12.78	12.62	0.11	0.112
2.0	15.78	15.86	15.86	15.83	0.04	0.170
2.5	10.91	11.32	11.14	11.12	0.17	0.080
3.0	14.13	14.42	14.29	14.28	0.12	0.140
3.5	12.99	12.80	12.76	12.85	0.10	0.115
4.0	9.51	9.63	9.44	9.53	0.01	0.050
4.5	10.68	10.58	10.56	10.61	0.05	0.075
5.0	10.23	10.35	10.33	10.30	0.05	0.065
5.5	11.72	11.77	11.90	11.80	0.07	0.095
6.0	11.96	12.14	12.10	12.07	0.08	0.100
6.5	14.12	14.39	14.29	14.27	0.11	0.140
7.0	10.36	10.80	10.55	10.57	0.18	0.070
7.5	16.20	16.41	16.26	16.29	0.09	0.180
8.0	10.58	10.55	10.25	10.46	0.15	0.070
8.5	10.26	10.48	10.33	10.36	0.09	0.065
9.0	8.85	8.95	8.82	8.87	0.06	0.040
9.5	9.80	10.01	9.99	9.93	0.09	0.060
10.0	7.97	7.98	7.92	7.96	0.03	0.025

Appendix A-19 cont'd.

Time	Fluorescence					SA conc.
(hrs)	1	2	3	Mean*	SD	(mg/ml)
10.5	11.47	11.64	11.69	11.60	0.09	0.090
11.0	11.50	11.42	11.30	11.41	0.08	0.085
11.5	8.41	8.53	8.40	8.45	0.06	0.030
12.0	11.10	10.96	10.84	10.97	0.11	0.078
24.0	9.72	9.61	9.80	9.71	0.06	0.054

*Dilution factor of 1000

Figure A-1. Standard curve for sodium salicylate fluorometric assay in gastric juice.

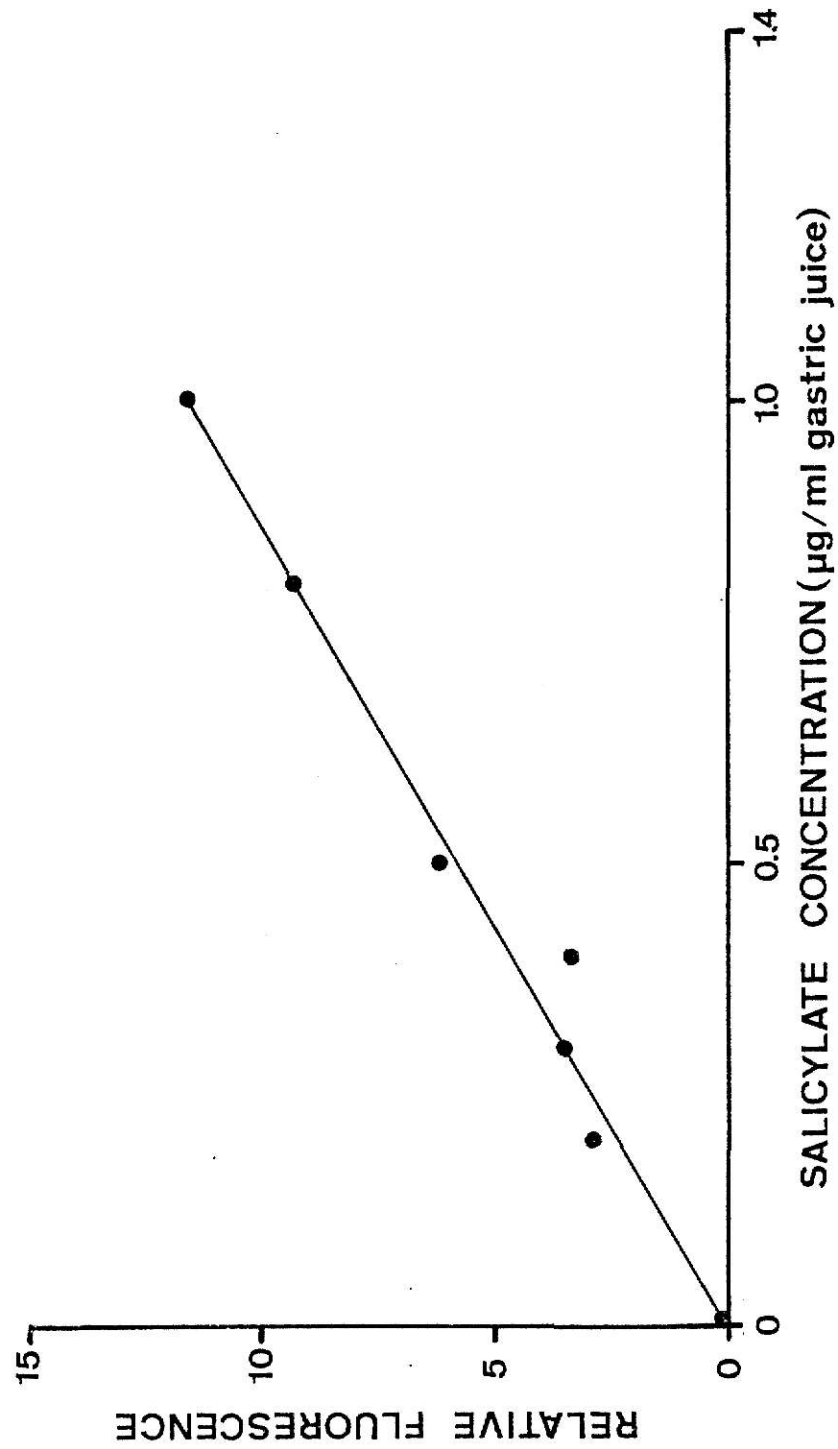
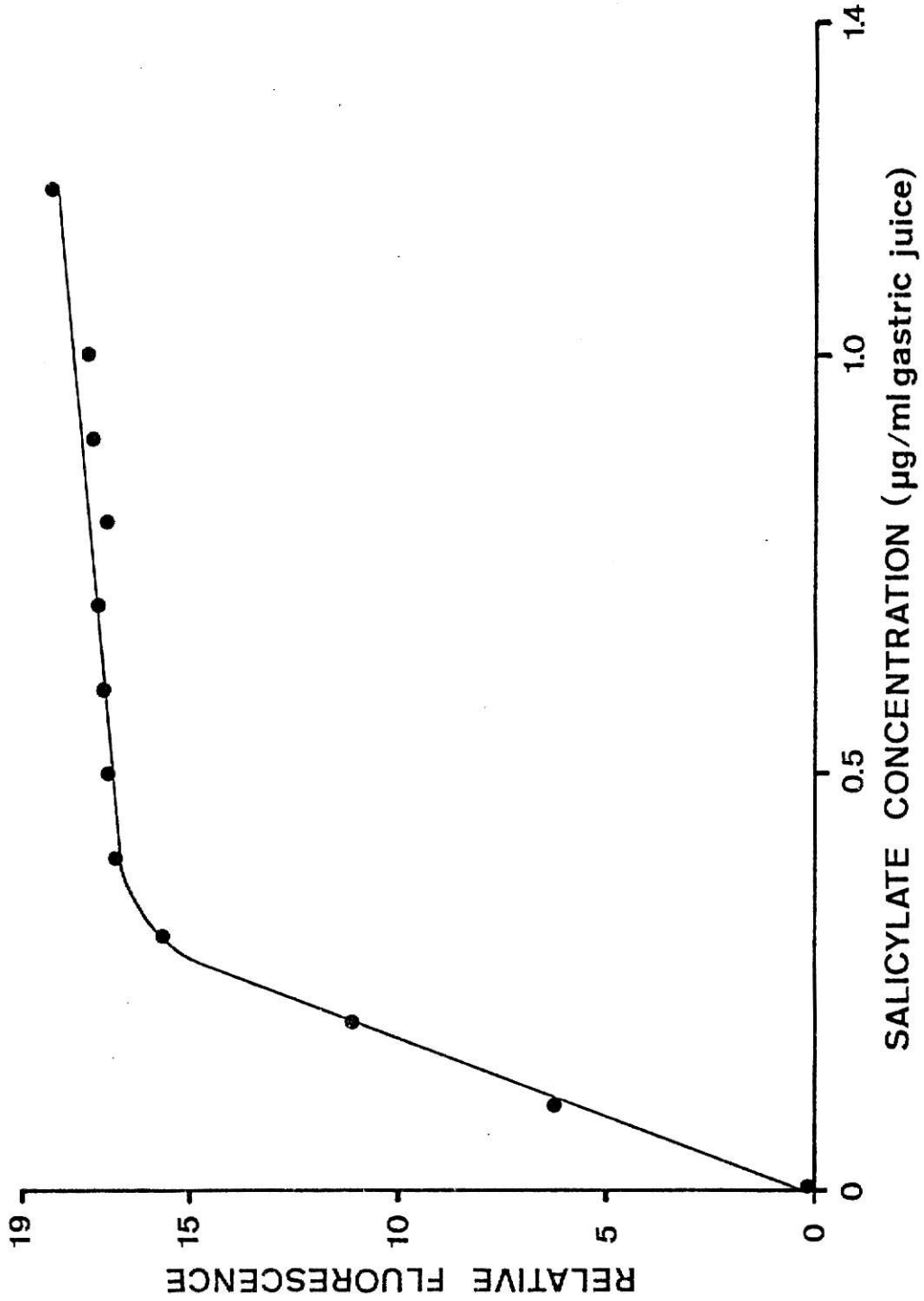

RELATIVE FLUORESCENCE
15
10
5
0
0
0.5
1.0
1.4
SALICYLATE CONCENTRATION (µg/ml gastric juice)

Figure A-2. Standard curve for sodium salicylate fluorometric assay in dog plasma.


RELATIVE FLUORESCENCE
19
15
10
5
0
0
0.5
1.0
1.4
SALICYLATE CONCENTRATION (µg/ml gastric juice)

INDIVIDUAL EXPERIMENTAL AND ANIMAL DATA

PROSTAGLANDIN-$F_{2\alpha}$ ($PGF_{2\alpha}$) STUDIES

Appendix B-1. Radioimmunoassay of $PGF_{2\alpha}$ from dog A plasma after dosing with 30 mg/kg salicylic acid as Excedrin.

Time (hrs)	Radioactivity (CPM) 1	2	Mean	DPM
0.0	40893	40763	40828	1168371
0.25	37446	37449	37448	1069886
0.5	36980	36982	36981	1056571
1.0	30689	30687	30688	873971
1.5	28694	28695	28695	819829
2.0	25533	25529	25531	729514
2.5	33003	33010	33007	942943
3.0	34753	34778	34766	992943
6.0	36577	36500	36317	1061514
12.0	37609	37606	37608	1074543
18.0	45472	45478	45475	1082667
24.0	38112	38113	38113	1088914

DPM = Disintegration per minute

CPM = Counts per minute

Appendix B-2. Radioimmunoassay of $PGF_{2\alpha}$ from dog B plasma after dosing with 30 mg/kg salicylic acid as Excedrin.

Time (hrs)	Radioactivity (CPM)			DPM
	1	2	Mean	
0.0	38650	38649	38650	1104286
0.25	35651	35660	35656	1018600
0.5	22316	22314	22315	637600
1.0	22062	22058	22060	630343
1.5	19670	19663	19667	562000
2.0	18193	18190	18192	519800
2.5	20375	20376	20376	582143
3.0	21758	21750	21754	621657
6.0	34999	35004	35002	999971
12.0	16261	16176	16220	102198
18.0	89540	89543	89542	1053494
24.0	36914	37410	37162	1054686

DPM = Disintegration per minute

CPM = Counts per minute

Appendix B-3. Radioimmunoassay of $PGF_{2\alpha}$ from dog A plasma after dosing with 30 mg/kg salicylic acid as Ascriptin.

Time (hrs)	Radioactivity (CPM)			DPM
	1	2	Mean	
0.0	40636	40640	40638	1161029
0.25	37609	37525	37567	1074543
0.5	36914	37410	37162	1054686
1.0	33141	33247	33194	946886
1.5	29507	29501	29504	843057
2.0	28165	28060	28113	804714
2.5	22272	22251	22262	635743
3.0	21095	20886	20991	602714
6.0	26316	26417	26367	754771
12.0	40442	40450	40446	962905
18.0	34275	34641	34458	989743
24.0	12700	13520	13110	1021857

DPM = Disintegration per minute

CPM = Counts per minute

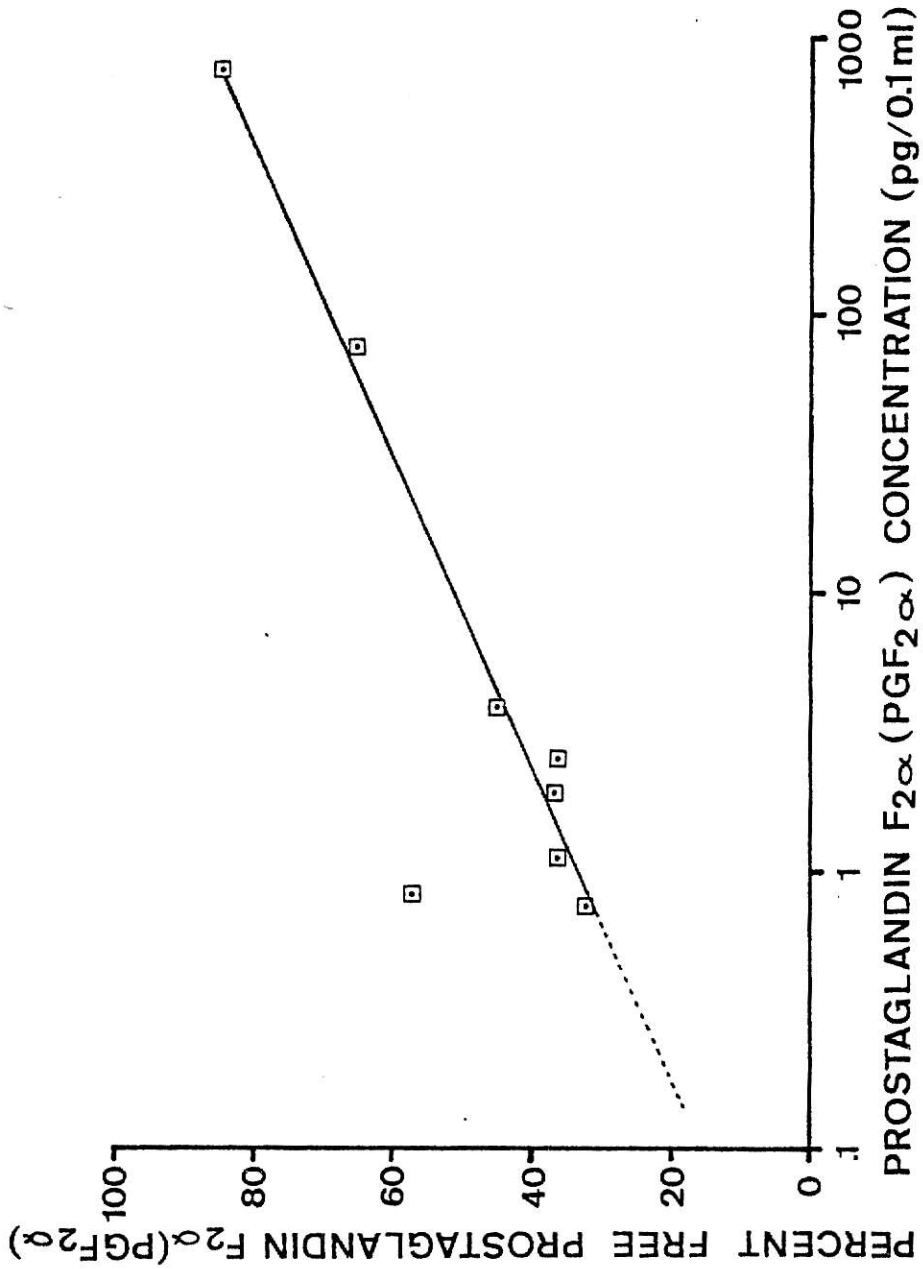
Appendix B-4. Radioimmunoassay of $PGF_{2\alpha}$ from dog B plasma after dosing with 30 mg/kg salicylic acid as Ascriptin.

Time (hrs)	Radioactivity (CPM)			
	1	2	Mean	DPM
0.0	29733	29734	29734	895684
0.25	89547	90303	89925	1053494
0.5	34275	34641	34458	989743
1.0	30179	30532	30356	872343
1.5	62848	64981	63915	764483
2.0	16469	16439	16454	270543
2.5	18738	18820	18779	535371
3.0	17620	17624	17622	498829
6.0	26936	27178	27057	776574
12.0	35811	36381	36096	859429
18.0	75830	75831	75831	892118
24.0	32351	32349	32350	924314

DPM = Disintegration per minute

CPM = Counts per minute

Figure B-1 Standard curve for prostaglandin-$F_{2\alpha}$ assay using dinoprost tromethamine.


PERCENT FREE PROSTAGLANDIN $F_{2\alpha}$($PGF_{2\alpha}$)
0
20
40
60
80
100
.1
1
10
100
1000
PROSTAGLANDIN $F_{2\alpha}$ ($PGF_{2\alpha}$) CONCENTRATION (pg/0.1 ml)

INVESTIGATION OF ACETYLSALICYLIC ACID BIOAVAILABILITY AND ITS EFFECT ON PROSTAGLANDIN-$F_{2\alpha}$ IN DOGS

by

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BSc., Ahmadu Bello University, 1975

AN ABSTRACT OF A MASTER'S THESIS

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Salicylates, which include salicylic acid, its esters, and derivatives, relieve pain, fever, and rheumatism because of their inhibitory effects on prostaglandin synthetase. We determined the rate of salicylate release from eleven commercial sources of aspirin in artificial gastric juice. The two most soluble brands of aspirin were then studied *in vivo* in dogs. Artificial gastric juice and plasma salicylate concentrations were measured by fluorescence spectrophotometry. Using a radioimmunoassay, we also measured prostaglandin ($PGF_{2\alpha}$) levels in the dogs after treatment with 30 mg salicylic acid/kg body weight from either Excedrin or Ascriptin.

Excedrin dissolved fastest of all the aspirin brands, followed by Ascriptin. The slowest dissolving brand was Excedrin P.M. Total plasma salicylate peaked about 2.5 hrs after dosing dogs orally with Excedrin and 3 hrs after dosing with Ascriptin. Excedrin left the plasma faster than did Ascriptin. Plasma salicylate concentrations due to Excedrin decreased to an average of 12 mg/ml in 6 hrs, while Ascriptin peaked at 3 hrs and remained there for more than 12 hrs.

Both Excedrin and Ascriptin reduced $PGF_{2\alpha}$ levels in plasma. Lowest concentrations of $PGF_{2\alpha}$ occurred 2 and 3 hrs after oral administration of Excedrin or Ascriptin, respectively. The percent free $PGF_{2\alpha}$ increased linearly to a maximum of 85% free $PGF_{2\alpha}$. The percent free $PGF_{2\alpha}$ decreased as total $PGF_{2\alpha}$ concentrations decreased.

This study documented a wide range of bioavailability among commercial aspirin brands. This was confirmed by additional studies in dogs. The effect of aspirin in depressing circulating $PGF_{2\alpha}$ levels in dog was observed via a radioimmune assay technique.